Quenchers Introduction

Quenchers are substances capable of absorbing energy from a fluorophore (such as a fluorescent dye) and re-emitting much of that energy as either heat (in the case of dark quenchers) or visible light (in the case of fluorescent quenchers). Dabcyl is an example of a dark quencher, and TAMRA is an example of a fluorescent quencher. When the fluorophore and quencher are in close proximity, the quencher absorbs the energy emitted from an excited fluorophore, thereby suppressing its emission. When the two substances are widely separated, the quencher no longer can absorb the fluorophore's emission, and the latter's presence can be visually detected (1). These properties are utilized in many popular oligonucleotide probes currently used for research or diagnostic purposes, such as TaqMan or Molecular Beacon probes (2-3). In such probes, a quencher and fluorophore having overlapping absorption and emission spectra, respectively, are incorporated into the probe as a pair. The probe is designed such that the quencher and fluorophore will always remain in close proximity if the specific target is not present, and be widely separated if it is present. Observation of a fluorescent signal thus indicates presence of target, and lack of a fluorescent signal indicates absence of target.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 **Black Hole Quencher License Agreement
"Black Hole Quencher", "BHQ-1", "BHQ-2" and "BHQ-3" are registered trademarks of Biosearch Technologies, Inc., Novato, CA.

Dye & Quencher Spectral Overlap

Quenchers Design Protocols

Selection of Dye-Quencher Combinations for Use in Fluorescent Hybridization Probes

The development of a wide variety of fluorescent dyes and quencher molecules for use as detection systems for nucleic acid hybridization probes has permitted the development of several different real-time PCR assay systems that utilize dye-quencher hybridization probes that produce a fluorescent signal only when they bind to their target. However, in order to properly design such an assay for a particular target(s) of interest, it is important to carefully select the dye and quencher of hybridization probes, based on the type of hybridization probe used in the assay, the number of targets to be detected, and the type of apparatus available to perform the assay.

1) Choose fluorescent dyes that can be properly excited and detected by the excitation source and the optics of the spectrophotometric thermal cycler you plan to use in your experiments. Laser-based excitation sources typically provide optimal excitation within a narrow wavelength range (e.g., 500-540 nm for argon ion lasers), while white light sources (e.g., tungsten-halogen lamp) and light-emitting diodes in combination with excitation and emission filters can excite fluorophores within the entire visible range (400-700 nm).

2) For detection of one target with one hybridization probe, FAM, HEX, or TET are recommended for the dye, since they can be detected on all spectrophotometric thermal cyclers, and phosphoramidites are available for all three (which makes synthesis of the probe straightforward and cost-effective).

3) For multiplexed assays, where at least two different hybridization probes are necessary, dyes with well-separated absorption and emission wavelength should be chosen.

4) For FRET hybridization probes, fluorescent dye-quencher pairs with a high degree of spectral overlap should be chosen. See Quencher Selection table to select appropriate quencher.

5) For contact quenching-based hybridization probes (e.g., molecular beacons), ANY non-fluorescent quencher should work well.

Dye & Quencher Selection Table

Dye & Quencher Selection Table

Quenchers Applications

Dark quenchers are typically used in fluorescent dye-quencher probes requiring suppression of the dye's fluorescence under one set of circumstances, but not under another, such as in the case of TaqMan and Molecular Beacons probes. Common examples of dark quenchers are Dabcyl and the three Black Hole Quenchers (BHQ). Taken together, the absorption spectra of these four dark quenchers span the entire visible range, which provides a researcher with broad flexibility in choice of fluorescent dye, along with the ability to search a sample for multiple targets in a multiplex quantitative PCR format. Fluorescent quenchers, such as TAMRA, are typically used in fluorescence resonance energy transfer (FRET)-based applications. FRET probes contain a donor (fluorescent dye)-acceptor (fluorescent quencher) pair in close proximity. After absorbance of light by the donor moiety, the donor's fluorescence emission energy is absorbed (quenched) by the acceptor moiety, and subsequently emitted at the acceptor's emission wavelength. The result is a final fluorescence emission at a substantially longer wavelength than would be expected if only the donor moiety were present (4). FRET probes thus are useful in cases where a substantial shift in final emission wavelength is desirable. This type of FRET-based system is typically used to determine intra- and inter-molecular distances at very high resolution (1-10 nm) (5). For example, FRET oligo probes have been used to measure the dynamic changes in intermolecular distances between tRNAs bound at the A and P sites of ribosomes during mRNA translation (6). FRET oligo primers have also been used to obtain direct evidence of strand slippage during in vitro synthesis of poly(dG)-poly(dC) duplexes by the Kleno exo- fragment of DNA polymerase I (7).

References

(1) Lakowicz, J.R. Quenching of Fluorescence. in Principles of Fluorescence Spectroscopy, 3rd Edition (2006), Springer-Verlag, Berlin, 278-330.
(2) Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.PCR Methods Appl. (1995), 4: 1-6.
(3) Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.
(4) Lakowicz, J.R. Quenching of Fluorescence. in Principles of Fluorescence Spectroscopy, 3rd Edition (2006), Springer-Verlag, Berlin, 331-352.
(5) Stryer, L., Haugland, R.P. Energy transfer: a spectroscopic ruler. Proc. Natl. Acad. Sci. USA (1967) 58: 719-726.
(6) Munro, J.B., Altman, R.B., O'Connor, N., Blanchard, S.C. Molecular Cell (2007), 25: 505-517.
(7) Kotlyar, A.B., Borovok, N., Molotsky, T., Fadeev, L., Gozin, M. In vitro synthesis of uniform poly(dG)-poly(dC) by Klenow exo(-) fragment of polymerase I. Nucleic Acids Res. (2005), 33: 525-535.

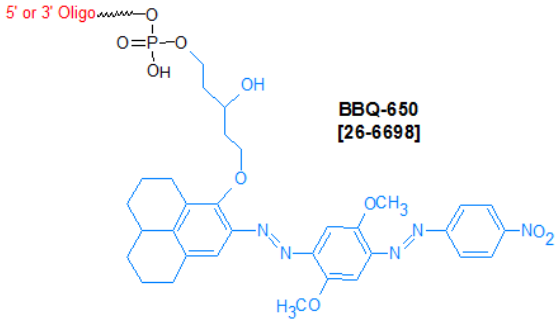
Modificaton Code List

Modification	Code	Catalog Number
3'-BBQ-650 (Black Berry Quencher 650)	[3-BBQ-650]	26-6698
BHQ-0 (Black Hole Quencher 0, 3')	[BHQ-0-3]	26-6475
BHQ-1 (Black Hole Quencher 1, 3')	[3-BHQ-1]	26-6472
BHQ-2 (Black Hole Quencher 2, 3')	[3-BHQ-2]	26-6468
BHQ-3 (Black Hole Quencher 3, 3')	[3-BHQ-3]	26-6473
BHQ-1 (Black Hole Quencher-1, 5')	[5-BHQ-1]	26-6727
BHQ-2 (Black Hole Quencher-2, 5')	[5-BHQ-2]	26-6728
BHQ-3 (Black Hole Quencher-3, 5')	[5-BHQ-3]	26-6729
Atto 540Q	[Atto540Q-N]	26-6962
Atto 575Q	[Atto575Q-N]	26-6969
Atto 612Q	[Atto612Q-N]	26-6975
BBQ-650 Internal	[BBQ-650-Int]	26-6776
BBQ-650 NHS (Black Berry Quencher 650 NHS)	[BBQ-650 N]	26-6734
BBQ-650-dT (Black Berry Quencher 650 dT)	[BBQ-650-dT]	26-6699
BHQ-1 Internal	[BHQ-1-Int]	26-6775
BHQ-1-NHS (Black Hole Quencher 1 NHS)	[BHQ-1 N]	26-6732
BHQ-1-dT (Black Hole Quencher 1 dT)	[BHQ-1-dT]	26-6652
BHQ-2 Internal	[BHQ-2-Int]	26-6772
BHQ-2-dT (Black Hole Quencher 2 dT)	[BHQ-2-dT]	26-6653
Dabcyl Quencher deoxythymidine dT	[Dab-dT]	26-6446

Dabcyl Quencher-3'	[Dab-3]	26-6470
Dabcyl-5'	[Dab-5]	26-6704
MGB 3' CDPI3	[MGB-CDPI3-3]	26-6456
MGB-5' CDPI3	[MGB-CDPI3-5]	26-6457
Tamra NHS	[Tamra-N]	26-6450
TAMRA-3' (Carboxytetramethylrhodamine)	[3-Tamra]	26-6451
Tamra-dT (Carboxytetramethylrhodamine-dT)	[Tamra-dT]	26-6449

3'-BBQ-650

Category	Quenchers
Modification Code	3-BBQ-650
Reference Catalog Number	26-6698
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	667.63



BlackBerry Quencher 650 (BBQ650) is classified as a dark quencher (a non-fluorescent chromophore). Dark quenchers are extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes in which the fluorophore has a long wavelength (yellow to far red) emission maximum (e.g. Cy3, ROX, Cy5, Cy 5.5). Dark quenchers can serve in this role because they have long wavelength absorbance maxima. Dark quenchers are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BBQ650 has an absorbance maximum of 650 nm, and an effective absorbance range of 550-750 nm (yellow to far red). It is chemically resistant to both oligonucleotide synthesis reagents (iodine, TCA) or deblocking solutions (ammonia, AMA). **Consequently, for synthesis of longer oligos (> 50 bases), BBQ650 is the preferred quencher over BHQ-2 or BHQ-3, as the latter are chemically less stable, and degrade when exposed to oligo synthesis and deprotection conditions for long periods of time, such as when synthesizing or processing longer oligos. <**

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

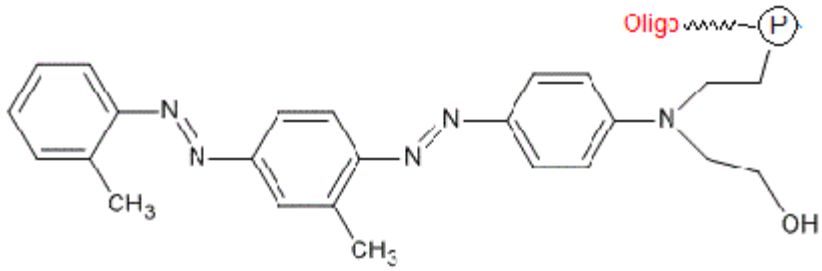
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

3'-BHQ-0

Category	Quenchers
Modification Code	BHQ-0-3
Reference Catalog Number	26-6475
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	479.47



Black Hole Quencher 0 (BHQ-0)
[26-6475-XX]

Black Hole Quencher-0 (BHQ-0) is classified as a dark quencher (a non-fluorescent chromophore), and is extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-0 has an absorbance maximum of 493 nm, and an effective absorbance range of 430-520 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in the Blue-green to yellow-green part of the visible range (430-520 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-1 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

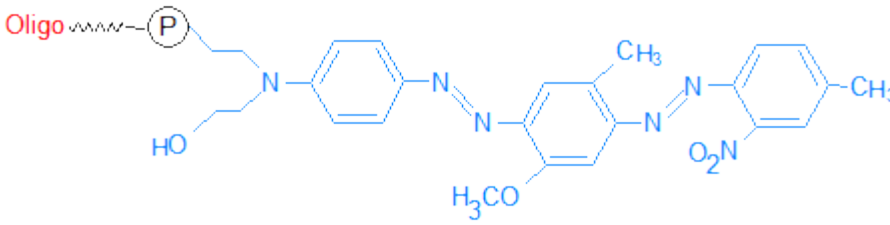
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

3'-BHQ-1

Category	Quenchers
Modification Code	3-BHQ-1
Reference Catalog Number	26-6472
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	554.49



Black Hole Quencher 1 (BHQ-1)
[26-6472-XX]

Black Hole Quencher-1 (BHQ-1) is classified as a dark quencher (a non-fluorescent chromophore), and is extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-1 has an absorbance maximum of 534 nm, and an effective absorbance range of 480-580 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in theyellow-green to yellow part of the visible range (519-556 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-1 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

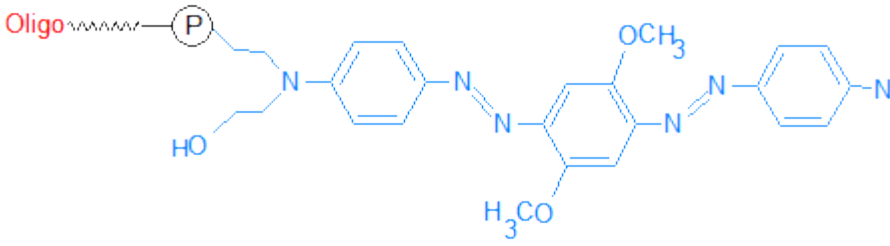
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

3'-BHQ-2

Category	Quenchers
Modification Code	3-BHQ-2
Reference Catalog Number	26-6468
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	556.47



Black Hole Quencher 2 (BHQ-2)
[26-6468-XX]

Black Hole Quencher-2 (BHQ-2) is classified as a dark quencher (a non-fluorescent chromophore), and is extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-2 has an absorbance maximum of 579 nm, and an effective absorbance range of 550-650 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in the yellow-orange part of the visible range (557-617 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-2 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

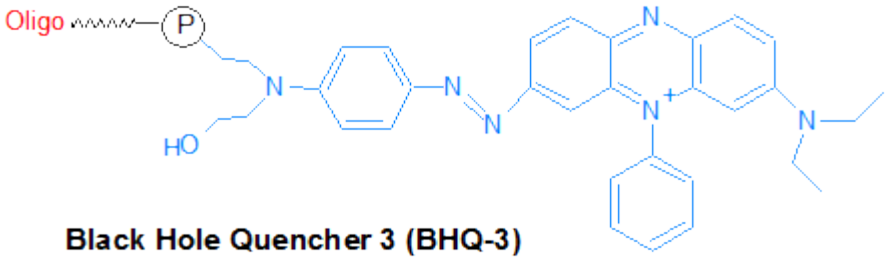
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

3'-BHQ-3

Category	Quenchers
Modification Code	3-BHQ-3
Reference Catalog Number	26-6473
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	597.63



Black Hole Quencher 3 (BHQ-3)
[26-6473-XX]

Black Hole Quencher-3 (BHQ-3) is classified as a dark quencher (a non-fluorescent chromophore), and is used as a quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-3 has an absorbance maximum of 672 nm, and an effective absorbance range of 620-730 nm. **BHQ-3, is chemically less stable, and degrades when exposed to oligo synthesis and deprotection conditions for long periods of time, such as when synthesizing or processing longer oligos. We recommend BBQ650, a dark quencher as an effective substitute. BBQ650 has an absorbance maximum of 650 nm, and an effective absorbance range of 550-750 nm (yellow to far red). It is chemically resistant to both oligonucleotide synthesis reagents, deblocking and deprotecting reagents that includes harsh chemicals, acid and ammonia solutions.**

BBQ650.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

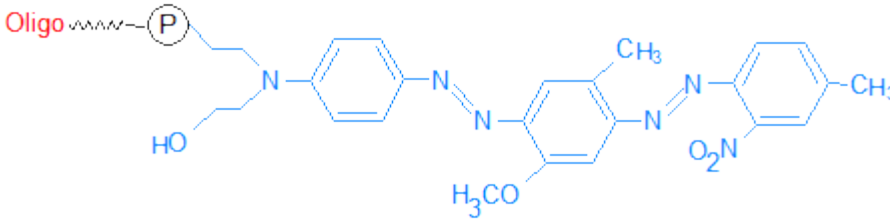
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

5'-BHQ-1

Category	Quenchers
Modification Code	5-BHQ-1
Reference Catalog Number	26-6727
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	538.47



Black Hole Quencher 1 (BHQ-1)
[26-6472-XX]

Black Hole Quencher-1 (BHQ-1) is classified as a dark quencher (a non-fluorescent chromophore), and is extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-1 has an absorbance maximum of 534 nm, and an effective absorbance range of 480-580 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in theyellow-green to yellow part of the visible range (519-556 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-1 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

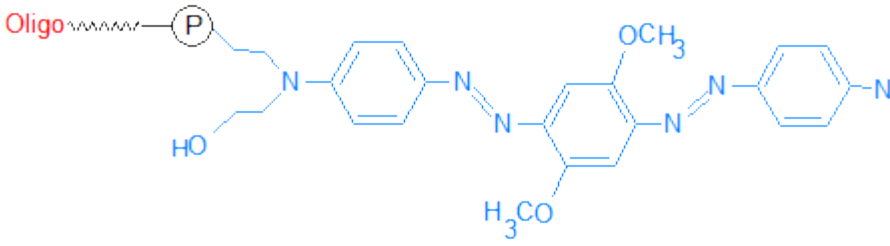
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

5'-BHQ-2

Category	Quenchers
Modification Code	5-BHQ-2
Reference Catalog Number	26-6728
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	556.5



Black Hole Quencher 2 (BHQ-2)
[26-6468-XX]

Black Hole Quencher-2 (BHQ-2) is classified as a dark quencher used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-2 has an absorbance maximum of 579 nm, and an effective absorbance range of 550-650 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in the yellow-orange part of the visible range (557-617 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-2 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

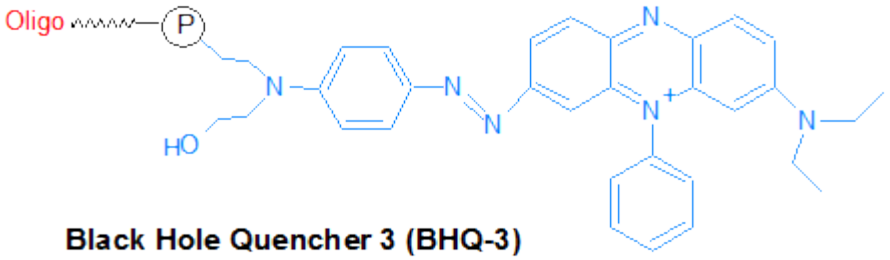
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

5'-BHQ-3

Category	Quenchers
Modification Code	5-BHQ-3
Reference Catalog Number	26-6729
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	597.63



Black Hole Quencher 3 (BHQ-3)
[26-6473-XX]

5'-BHQ-3 has been discontinued. We recommend BBQ650 as a substitute. **BBQ650.**

Black Hole Quencher-3 (BHQ-3) is classified as a dark quencher (a non-fluorescent chromophore), and is used as a quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-3 has an absorbance maximum of 672 nm, and an effective absorbance range of 620-730 nm. **BHQ-3, is chemically less stable, and degrades when exposed to oligo synthesis and deprotection conditions for long periods of time, such as when synthesizing or processing longer oligos. We recommend BBQ650, a dark quencher as an effective substitute. BBQ650 has an absorbance maximum of 650 nm, and an effective absorbance range of 550-750 nm (yellow to far red). It is chemically resistant to both oligonucleotide synthesis reagents, deblocking and deprotecting reagents that includes harsh chemicals, acid and ammonia solutions.**

BBQ650.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

Atto 540Q

Category	Fluorescent Dyes
Modification Code	Atto540Q-N
Reference Catalog Number	26-6962
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	756

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined.

ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Atto 575Q

Category	Fluorescent Dyes
Modification Code	Atto575Q-N
Reference Catalog Number	26-6969
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	787

Atto 580Q has been replaced by Atto 575Q.
ATTO 575Q is a novel fluorescence quencher (energy acceptor in FRET process). Characteristic features of the label are strong absorption and high thermal and photo-stability. The dye is moderately hydrophilic. ATTO 575Q is a cationic dye. Contrary to ATTO 580Q, ATTO 575Q is supplied as single isomer. After coupling to a substrate the dye carries a net electrical charge of +1.
ATTO 575Q has an absorption maximum at 582 nm (H2O). Atto 580Q is characterized by a high photostability and thermostability. Ato 575Q can be utilized as a fluorescence quencher (λ absorbance = 582 nm) on amine-labeled nucleotides for FRET experiments. Works well in combination with the following fluorescent dyes: TAMRA, ATTO 550, ATTO 565, ATTO 590, and ROX.

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection.
ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined.

ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Atto 612Q

Category	Fluorescent Dyes
Modification Code	Atto612Q-N
Reference Catalog Number	26-6975
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	888

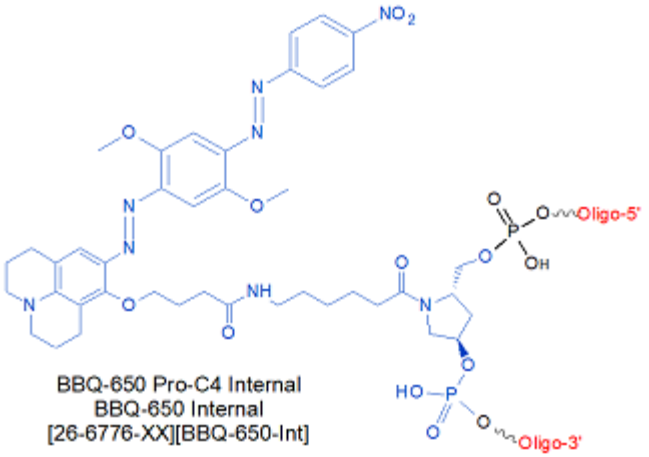
This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined.

ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

BBQ-650 Internal

Category	Quenchers
Modification Code	BBQ-650-Int
Reference Catalog Number	26-6776
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	862.9



BBQ-650 Pro-C4 Internal
BBQ-650 Internal
[26-6776-XX][BBQ-650-Int]

BlackBerry Quencher 650 (BBQ650) is classified as a dark quencher (a non-fluorescent chromophore). Dark quenchers are extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes in which the fluorophore has a long wavelength (yellow to far red) emission maximum (e.g. Cy3, ROX, Cy5, Cy 5.5). Dark quenchers can serve in this role because they have long wavelength absorbance maxima. Dark quenchers are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BBQ650 has an absorbance maximum of 650 nm, and an effective absorbance range of 550-750 nm (yellow to far red). It is chemically resistant to both oligonucleotide synthesis reagents (iodine, TCA) or deblocking solutions (ammonia, AMA). **Consequently, for synthesis of longer oligos (> 50 bases), BBQ650 is the preferred quencher over BHQ-2 or BHQ-3, as the latter are chemically less stable, and degrade when exposed to oligo synthesis and deprotection conditions for long periods of time, such as when synthesizing or processing longer oligos.**
A list of specific fluorescent dyes compatible with BBQ650 is found at this link.
Click here for complete list of quenchers
The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BBQ650 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using BBQ650 as a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes (due to BBQ650 being resistant to degradation during the oligo deprotection step). <

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

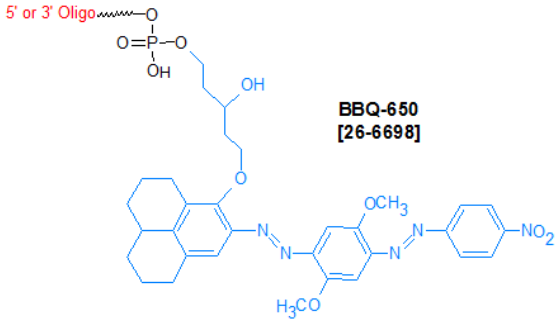
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

BBQ-650 NHS

Category	Quenchers
Modification Code	BBQ-650 N
Reference Catalog Number	26-6734
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	667.63



NHS modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis

BlackBerry Quencher 650 (BBQ650) is classified as a dark quencher (a non-fluorescent chromophore). Dark quenchers are extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes in which the fluorophore has a long wavelength (yellow to far red) emission maximum (e.g. Cy3, ROX, Cy5, Cy 5.5). Dark quenchers can serve in this role because they have long wavelength absorbance maxima. Dark quenchers are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BBQ650 has an absorbance maximum of 650 nm, and an effective absorbance range of 550-750 nm (yellow to far red). It is chemically resistant to both oligonucleotide synthesis reagents (iodine, TCA) or deblocking solutions (ammonia, AMA). **Consequently, for synthesis of longer oligos (> 50 bases), BBQ650 is the preferred quencher over BHQ-2 or BHQ-3, as the latter are chemically less stable, and degrade when exposed to oligo synthesis and deprotection conditions for long periods of time, such as when synthesizing or processing longer oligos.**

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

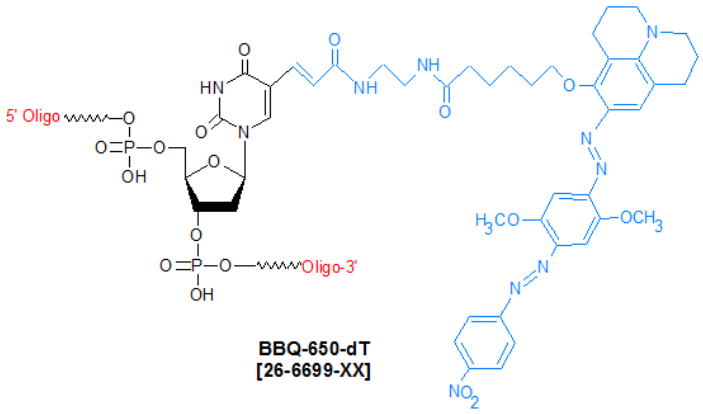
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

BBQ-650-dT

Category	Quenchers
Modification Code	BBQ-650-dT
Reference Catalog Number	26-6699
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	1000.95



BBQ650 dT can be used as a quencher for any internal position of the oligo.

BlackBerry Quencher 650 (BBQ650) is classified as a dark quencher (a non-fluorescent chromophore). Dark quenchers are extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes in which the fluorophore has a long wavelength (yellow to far red) emission maximum (e.g. Cy3, ROX, Cy5, Cy 5.5). Dark quenchers can serve in this role because they have long wavelength absorbance maxima. Dark quenchers are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BBQ650 has an absorbance maximum of 650 nm, and an effective absorbance range of 550-750 nm (yellow to far red). It is chemically resistant to both oligonucleotide synthesis reagents (iodine, TCA) or deblocking solutions (ammonia, AMA). **Consequently, for synthesis of longer oligos (> 50 bases), BBQ650 is the preferred quencher over BHQ-2 or BHQ-3, as the latter are chemically less stable, and degrade when exposed to oligo synthesis and deprotection conditions for long periods of time, such as when synthesizing or processing longer oligos.** A list of specific fluorescent dyes compatible with BBQ650 is found at this URL: (http://www.umass.edu/research/genomics/files/Which_Quencher.pdf). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BBQ650 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using BBQ650 as a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes (due to BBQ650 being resistant to degradation during the oligo deprotection step).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

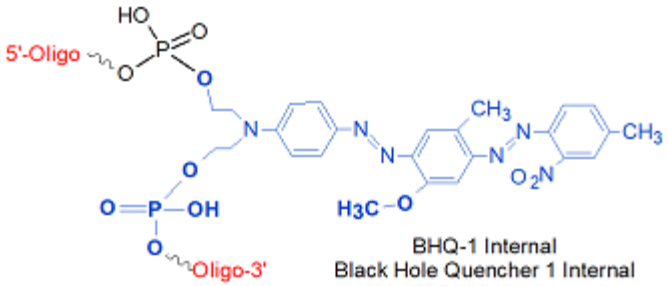
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

BHQ-1 Internal

Category	Quenchers
Modification Code	BHQ-1-Int
Reference Catalog Number	26-6775
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	538.47



BHQ-1 Internal
Black Hole Quencher 1 Internal
[26-6775-XX] [BHQ-1-Int]

Black Hole Quencher-1 (BHQ-1) is classified as a dark quencher (a non-fluorescent chromophore), and is extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-1 has an absorbance maximum of 534 nm, and an effective absorbance range of 480-580 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in theyellow-green to yellow part of the visible range (519-556 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-1 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

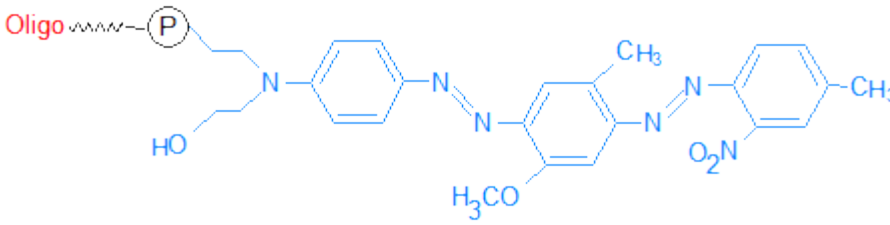
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

BHQ-1 NHS

Category	Quenchers
Modification Code	BHQ-1 N
Reference Catalog Number	26-6732
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	554.49



Black Hole Quencher 1 (BHQ-1)
[26-6472-XX]

NHS modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis

Black Hole Quencher-1 (BHQ-1) is classified as a dark quencher (a non-fluorescent chromophore), and is extensively used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-1 has an absorbance maximum of 534 nm, and an effective absorbance range of 480-580 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in theyellow-green to yellow part of the visible range (519-556 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-1 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

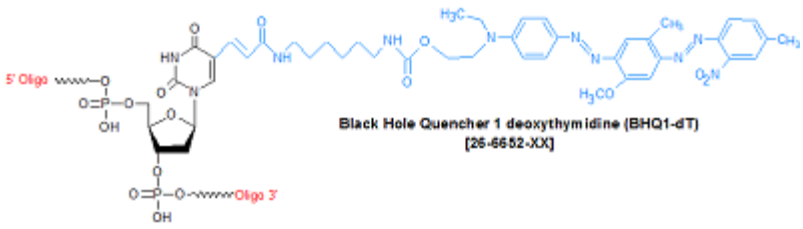
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

BHQ-1-dT

Category	Quenchers
Modification Code	BHQ-1-dT
Reference Catalog Number	26-6652
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	960.93



Black Hole Quencher-1 deoxythymidine (BHQ1-dT) is classified as a dark quencher (a non-fluorescent chromophore) nucleotide base, and is typically used to internally label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety. Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching. Other basic technical information about BHQ-1 is found in the BHQ-1 technical sheet.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

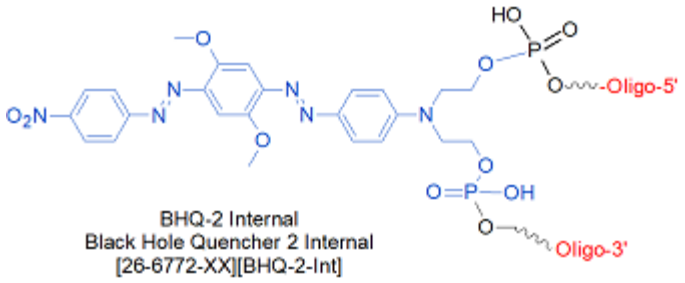
Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details **Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher BHQ^{R}, CAL Fluor^{R} and Quasar^{R} are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only.

Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

BHQ-2 Internal

Category	Quenchers
Modification Code	BHQ-2-Int
Reference Catalog Number	26-6772
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	556.5



BHQ-2 Internal
Black Hole Quencher 2 Internal
[26-6772-XX][BHQ-2-Int]

Black Hole Quencher-2 (BHQ-2) is classified as a dark quencher used as the 3'-quencher moiety in a variety of Fluorescence Resonance Energy Transfer (FRET) DNA detection probes. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

BHQ-2 has an absorbance maximum of 579 nm, and an effective absorbance range of 550-650 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in the yellow-orange part of the visible range (557-617 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-2 to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5).

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

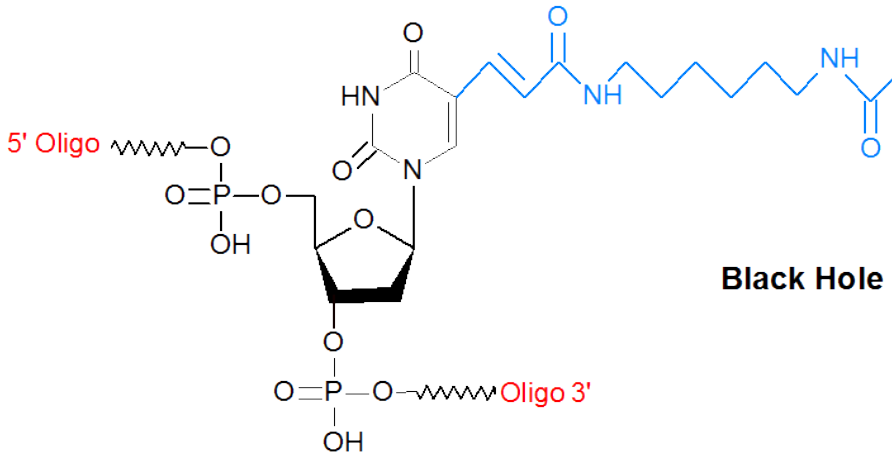
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

BHQ-2-dT

Category	Quenchers
Modification Code	BHQ-2-dT
Reference Catalog Number	26-6653
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	960.93



Black Hole

Black Hole Quencher-2 deoxythymidine (BHQ2-dT) is classified as a dark quencher (a non-fluorescent chromophore) nucleotide base, and is typically used to internally label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety. Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching. Other basic technical information about BHQ-2 is found in the BHQ-2 technical sheet.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

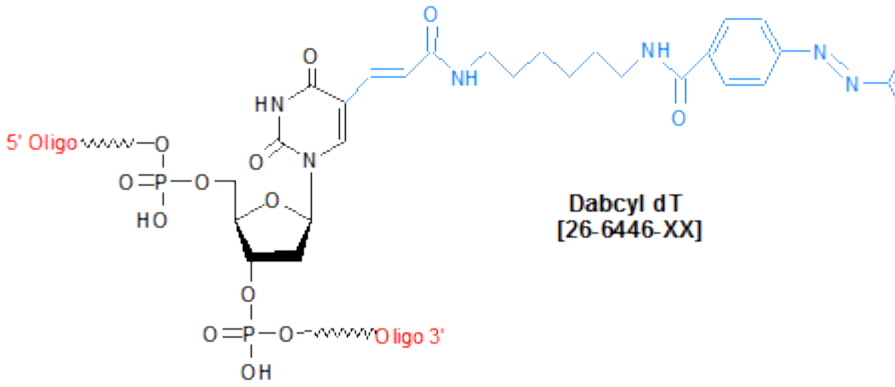
Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only.

Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

Dabcyl dT

Category	Quenchers
Modification Code	Dab-dT
Reference Catalog Number	26-6446
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	709.7



Dabcyl deoxythymidine (Dabcyl-dT) is classified as a dark quencher (a non-fluorescent chromophore) nucleotide base, and is typically used to internally label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety. Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

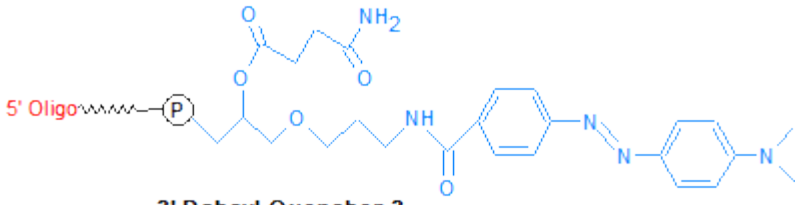
Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc.

"

Dabcyl-3'

Category	Quenchers
Modification Code	Dab-3
Reference Catalog Number	26-6470
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	462.44



3' Dabcyl Quencher-3
[26-6470-XX]

Dabcyl-3' is classified as a dark quencher (a non-fluorescent chromophore), and is typically used to label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety at the 3'-end. Such probes are primarily used in nucleic acid assays, but also find a place in nucleic acid structural studies (1). Examples include TaqMan probes (2), Scorpion primers (3), and Molecular Beacons (4).

Dabcyl-3' has an absorbance maximum of 479 nm, and an effective absorbance range of 346-489 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in the blue to green part of the visible range (442-506 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of Dabcyl to allow the latter to quench the fluorescence of the former with a high degree of efficiency.

The advantages of using a dark quencher in a FRET probe are (a) low background fluorescence (and thus better signal-to-noise ratio), (b) higher dynamic range, (c) amenability to multiplex assays (due to a dark quencher having no secondary fluorescence), and (d) ease of synthesis of FRET probes with a dark quencher (due to dark quenchers being resistant to degradation during the oligo deprotection step) (5). <

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750

genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=15">Click here for complete list of quenchers and details **Black Hole Quencher License Agreement

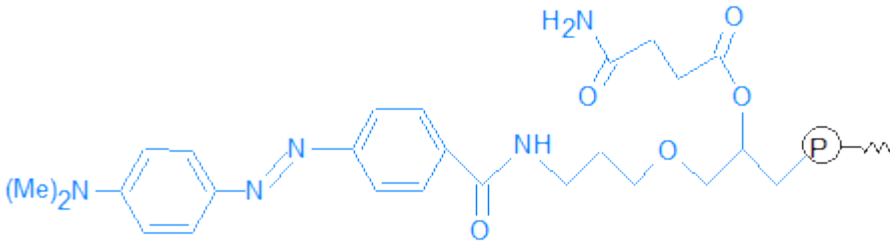
Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
5. Yeung, A.T., Holloway, B.P., Adams, P.S., Shipley, G.L. Evaluation of dual-labeled fluorescent DNA probe purity versus performance in real-time PCR. *Biotechniques.* (2004), **36**: 266-270, 272, 274-275.

Dabcyl-5'

Category	Quenchers
Modification Code	Dab-5
Reference Catalog Number	26-6704
5 Prime	Y
3 Prime	Y
Internal	N
Molecular Weight(mw)	462.44



Dabcyl-5'
[26-6704-XX]

Dabcyl-55 is classified as a dark quencher (a non-fluorescent chromophore), and is typically used to label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety at the 5'-end. TaqMan or Molecular Beacon probes labeled with Dabcyl at the 5'-end usually would be labeled with an appropriate fluorescent dye at the 3'-end; other kinds of FRET probes might have the fluorescent dye at an internal position. 5'-end labeling such probes with Dabcyl is unusual. The primary reason for using this inverse-labeling strategy is to take advantage of Dabcyl's very high hydrophobicity (which is higher than that of a fluorescent dye) to improve subsequent purification of the probe by HPLC or RPC. For example, a molecular beacon with 5'-Dabcyl and 3'-6-FAM is easier to purify (and will subsequently be purer) than a probe with the more common, opposite set-up.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details **Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher®, BHQ®, CAL Fluor® and Quasar® are registered trademarks of Biosearch Technologies, Inc.

, Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

MGB 3' CDPI3

Category	Duplex Stability
Modification Code	MGB-CDPI3-3
Reference Catalog Number	26-6456
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	813.27

MGB Probe Design: 5'-Fluorphore 5'-[Fluorophore]...probe sequence...[internal quencher][MGB]-3'
In the 5'-Fluorphore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed before the MGB at the 3' end.

MGB Probe Design: 3'-Fluorphore 5'-[MGB] [internal quencher]...probe sequence...[Fluorophore-3'
In the 3'-Fluorphore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed after the MGB at the 5' end.

MGB Probe Pricing MGB probe pricing is the total of the price for [MGB] + [internal quencher]+..probe sequence +[Fluorophore]-3'

The tripeptide of dihydropyrroloindole-carboxylate (CDPI3) is a minor groove binding (MGB) moiety derived from the natural product CC-1065 with strong DNA binding properties. Synthetic oligonucleotides with covalently-attached CDPI3 have enhanced DNA affinity and have improved the hybridization properties of sequence-specific DNA probes. Short CDPI3-oligonucleotides hybridize with single-stranded DNA to give more stable DNA duplexes than unmodified ODNs of similar length. CDPI3 MGβ-oligonucleotide conjugates have been found to be useful in the following applications:
- Arrest of primer extension and PCR blockers
- Short and fluorogenic PCR primers
- Real-time PCR probes
- miRNA Inhibitors

The simplest approach to MGB probe design is to use an MGB support, add a quencher molecule as the first addition and complete the synthesis with a 5'-fluorophore. Alternatively, a fluorophore support could be used with the 5' terminus containing a quencher molecule followed by a final MGB addition at the 5' terminus.
NOTICE: This product is sold under licensing arrangements between ELITechGroup Inc. and Glen Research. The purchase price of this product includes limited, nontransferable rights to use the product solely for activities of the purchaser which are directly related to human diagnostics. Other uses, including incorporation of the product into another commercial product, are prohibited without additional license rights. For information on purchasing a license to this product for purposes other than those stated above, contact: ELITech Group Molecular Diagnostics, 21720 23rd Drive SE, Suite 150, Bothell, WA 98021. Phone (425) 482-5555. Fax (425) 482-5550. Email: mdx@elitechgroup.com. This limited license permits the person or legal entity to which this product has been provided to use the product, and the data generated by use of the product, only for human diagnostics.

Neither ELITechGroup Inc. nor its licensors grants any other licenses, expressed or implied for any other purposes. Some components of nucleic acid analysis, such as specific methods and compositions for manipulating or visualizing nucleic acids for analysis, may be covered by one or more patents owned by other parties. Similarly, nucleic acids containing specific nucleotide sequences may be patented. Making, using, offering for sale, or selling such components or nucleic acids may require one or more licenses. Nothing in this document should be construed as an authorization or implicit license to make, use or sell any so covered component or nucleic acid under any such patents.

MGB-5' CDPI3

Category	Duplex Stability
Modification Code	MGB-CDPI3-5
Reference Catalog Number	26-6457
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	872.96

MGB Probe Design: 5'-Fluorphore 5'-[Fluorophore]...probe sequence...[internal quencher][MGB]-3'
In the 5'-Fluorphore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed before the MGB at the 3' end.

MGB Probe Design: 3'-Fluorphore 5'-[MGB] [internal quencher]...probe sequence...[Fluorophore-3'
In the 3'-Fluorphore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed after the MGB at the 5' end.

MGB Probe Pricing MGB probe pricing is the total of the price for [MGB] + [internal quencher]+..probe sequence +[Fluorophore]-3'

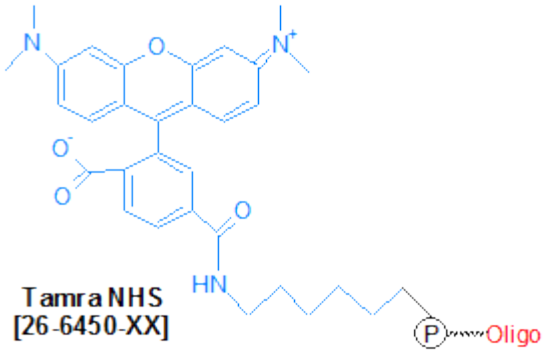
The tripeptide of dihydropyrroloindole-carboxylate (CDPI3) is a minor groove binding (MGB) moiety derived from the natural product CC-1065 with strong DNA binding properties. Synthetic oligonucleotides with covalently-attached CDPI3 have enhanced DNA affinity and have improved the hybridization properties of sequence-specific DNA probes. Short CDPI3-oligonucleotides hybridize with single-stranded DNA to give more stable DNA duplexes than unmodified ODNs of similar length. CDPI3 MGβ-oligonucleotide conjugates have been found to be useful in the following applications:
- Arrest of primer extension and PCR blockers
- Short and fluorogenic PCR primers
- Real-time PCR probes
- miRNA Inhibitors

The simplest approach to MGB probe design is to use an MGB support, add a quencher molecule as the first addition and complete the synthesis with a 5'-fluorophore. Alternatively, a fluorophore support could be used with the 5' terminus containing a quencher molecule followed by a final MGB addition at the 5' terminus.
NOTICE: This product is sold under licensing arrangements between ELITechGroup Inc. and Glen Research. The purchase price of this product includes limited, nontransferable rights to use the product solely for activities of the purchaser which are directly related to human diagnostics. Other uses, including incorporation of the product into another commercial product, are prohibited without additional license rights. For information on purchasing a license to this product for purposes other than those stated above, contact: ELITech Group Molecular Diagnostics, 21720 23rd Drive SE, Suite 150, Bothell, WA 98021. Phone (425) 482-5555. Fax (425) 482-5550. Email: mdx@elitechgroup.com. This limited license permits the person or legal entity to which this product has been provided to use the product, and the data generated by use of the product, only for human diagnostics.

Neither ELITechGroup Inc. nor its licensors grants any other licenses, expressed or implied for any other purposes. Some components of nucleic acid analysis, such as specific methods and compositions for manipulating or visualizing nucleic acids for analysis, may be covered by one or more patents owned by other parties. Similarly, nucleic acids containing specific nucleotide sequences may be patented. Making, using, offering for sale, or selling such components or nucleic acids may require one or more licenses. Nothing in this document should be construed as an authorization or implicit license to make, use or sell any so covered component or nucleic acid under any such patents.

Tamra NHS

Category	Fluorescent Dyes
Modification Code	Tamra-N
Reference Catalog Number	26-6450
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	527.53

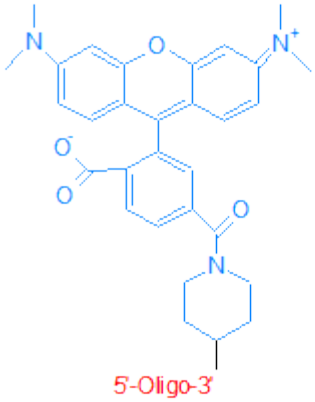


Tamra NHS
[26-6450-XX]

Carboxytetramethylrhodamine (TAMRA) is a fluorescent dye that is a derivative of rhodamine, and is used to label oligonucleotides at the 5'- or 3'-ends, or internally. TAMRA has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-modified oligonucleotides play a particularly important role in both fluorescence resonance energy transfer (FRET) and real-time PCR applications.

Tamra-3'

Category	Fluorescent Dyes
Modification Code	3-Tamra
Reference Catalog Number	26-6451
5 Prime	I
3 Prime	Y
Internal	I
Molecular Weight(mw)	527.53



3'-TAMRA (Carboxytetramethylrhodamine) [26-6451-XX]

Carboxytetramethylrhodamine (TAMRA) is a fluorescent dye that is a derivative of rhodamine, and is used to label oligonucleotides at the 5' or 3' ends, or internally. TAMRA has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-modified oligonucleotides play a particularly important role in both fluorescence resonance energy transfer (FRET) and real-time PCR applications.

FRET is a distance-dependent interaction between two dye molecules in which excitation is radiationlessly transferred from one dye (the donor) to the second dye (the acceptor), due to spectral overlap. Because the efficiency of the energy transfer is extremely sensitive to the distance between the molecules (varying as the inverse sixth power of that distance) (1), FRET can be used to study biological phenomena that produce changes in molecular proximity (2). For oligonucleotides slated for use in FRET application, a common donor-acceptor pair is 6-FAM (donor) / TAMRA (acceptor), due to their good spectral overlap. As the donor, 6-FAM is excited at 492 nm and transfers this energy to TAMRA, which then emits light at 580 nm. FRET oligo probes are widely used to monitor biochemical reactions, particularly in in vivo studies (3).

Besides being used as a FRET fluorophore, TAMRA also can be used as a FRET-based quencher moiety in real-time PCR probes such as TaqMan probes (4), Scorpion primers (5) and Molecular Beacons (6). For such probes, 6-FAM is used as the reporter moiety, and its emission at 521 nm is monitored. When 6-FAM and TAMRA are in close proximity, the former's fluorescence at 521 nm is quenched by the latter. After sufficient spatial separation of the two dyes during the course of the assay, 6-FAM's fluorescence is no longer quenched, and its fluorescence signal becomes observable.

TAMRA also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TAMRA at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

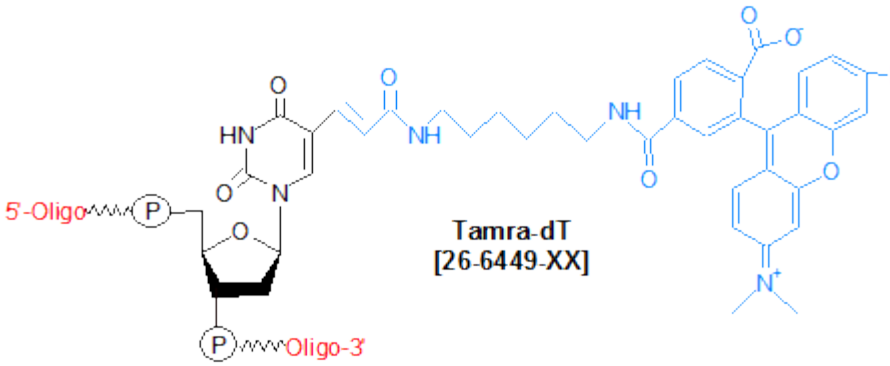
Note that, because TAMRA is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite (for internal labeling). The TAMRA-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.

References

1. Stryer, L., Haugland, R.P. Energy transfer: a spectroscopic ruler.*Proc. Natl. Acad. Sci. USA* (1967), **58**: 719-726.
2. Wu, P., Brand, L. Resonance energy transfer: methods and applications.*Anal. Biochem.* (1994), **218**: 1-13.
3. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications.*Biotechniques* (2001), **31**: 1106-1121.
4. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
5. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
6. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

Tamra-dT

Category	Fluorescent Dyes
Modification Code	Tamra-dT
Reference Catalog Number	26-6449
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	870.85



Carboxytetramethylrhodamine-deoxythymidien (TAMRA-dT) is a deoxythymidine nucleoside derivitized with TAMRA through a spacer arm. TAMRA-dT is used to internally label an oligonucleotide at a dT position. TAMRA-dT has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-dT can be used to internally label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety. Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching. For such probes, 6-FAM is most commonly used as the reporter moiety as the two dyes have excellent spectral overlap.

TAMRA-dT also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos internally labeled with TAMRA-dT also can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. For further details concerning the TAMRA dye, please see the technical sheet for it.