Black Hole Quencher-3 (BHQ-3) 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-3 has an absorbance maximum of 680 nm, and an effective absorbance range of 620-730 nm. It is the preferred quencher for pairing with fluorescent dyes that emit in the red-far red part of the visible range (640-775 nm). The emission spectra of this set of dyes sufficiently overlaps the absorbance spectrum of BHQ-3 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).

Quencher Spectral Data

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References