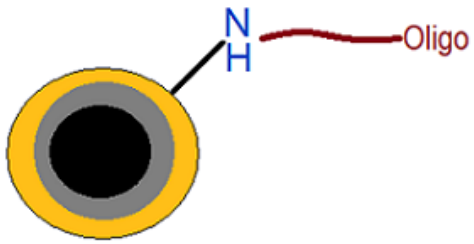
Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos,
Fluorescent dye labeled oligos, Molecular Beacons, siRNA, phosphonates
Fluorescent Probes; 2'-5' linked Oligos

Magnetic Beads Oligo Conjugation

For research use only. Not for use in diagnostic procedures for clinical purposes.

Reference Catalog Number	**26-7015**
Category	**Conjugation Chemistry**
Modification Code	**[Mag-Oligo]**
5 Prime	**Y**
3 Prime	**Y**
Internal	**Y**



Magentic bead [26-7015-XX] [Mag-Oligo]

Yield

1 micromolar scale: ~5 mg magnetic bead conjugated to ~2 nmol oligo. 10mg/mL suspension.
2 micromolar scale: ~10 mg magnetic bead conjugated to ~5 nmol oligo. 10mg/mL suspension.

Introduction

Gene Link offers a wide variety of modifications that enable oligonucleotides to be conjugated to various ligands including solid surfaces with the appropriate functional groups. Immuno chemistry based affinity ligands offered by Gene Link are custom oligos modified with Biotin and Digoxigenin. Gene Link offers magnetic beads conjugation for direct hybridization based affinity to complementary sequences. The most common conjugation chemistry involves oligos modified with a primary amino group; the amino group can be placed at 5' or 3' end or any desired internal position using an amino C6 base. The amino group reacts with *N*-hydroxysuccinimide (NHS) functional groups to form a stable amide linkage; the reaction efficiency varies from 50% to 90% depending on the ligand activated NHS group. The requirement for this conjugation is the availability of the desired ligand or solid surface in an activated NHS form for conjugation to the amino modified oligonucleotide.

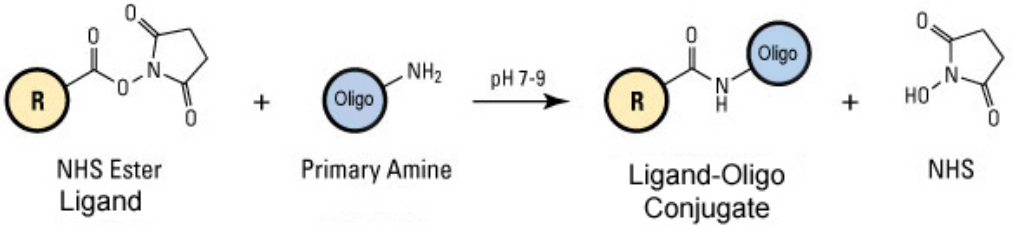
Material Supplied & Handling

1. The Oligo-Magnetic bead conjugation is varies between 0.3-0.5 nmol amine oligo/mg magnetic bead. Refer to specification sheet supplied with product and label on the tube.
2. The conjugated oligo-magnetic bead is supplied in High Salt Buffer (0.5 M NaCl, 50 mM Tris-HCl pH 7.5 and 0.02% sodium azide).
3. Wear appropriate personal protective equipment and clothing including lab coat, safety glasses and gloves.
4. Refer to the oligo specification sheet for the exact nmol oligo/mg of magnetic bead.
5. Sodium Azide Note: Dilute solutions of sodium azide are used in research laboratories as a preservative. This use generally presents no extraordinary dangers to the user, but it should be noted that weak solutions of sodium azide (0.1 to 1.0%) are eye and skin irritants.

Storage

DO NOT FREEZE. DO NOT CENTRIFUGE. Store the supplied oligo-magnetic bead at 4°C.

Amino Oligo Conjugation to NHS Ester Ligand



Magnetic Beads & Oligo Conjugation Characteristics	
Bead Size	1µm diameter
Number of Beads	~1.7 x 10^8 beads/mg
Surface Area	~100 m^2/g
Magnetization	~40 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Stability	pH 4-10
Binding Capacity	~1 nmol primary amine oligo/mg
Oligo Conjugate	0.3 to 0.5 nmol primary amine oligo/mg
Storage	Store at 4°C upon receipt.

The activated magnetic beads contain *N*-hydroxysuccinimide (NHS) functional groups, which react with primary amine modification on the oligonucleotide. The reaction efficiency is rarely above 50% thus the final conjugation varies from 0.3 to 0.5 nmol of oligo/mg of magnetic bead.

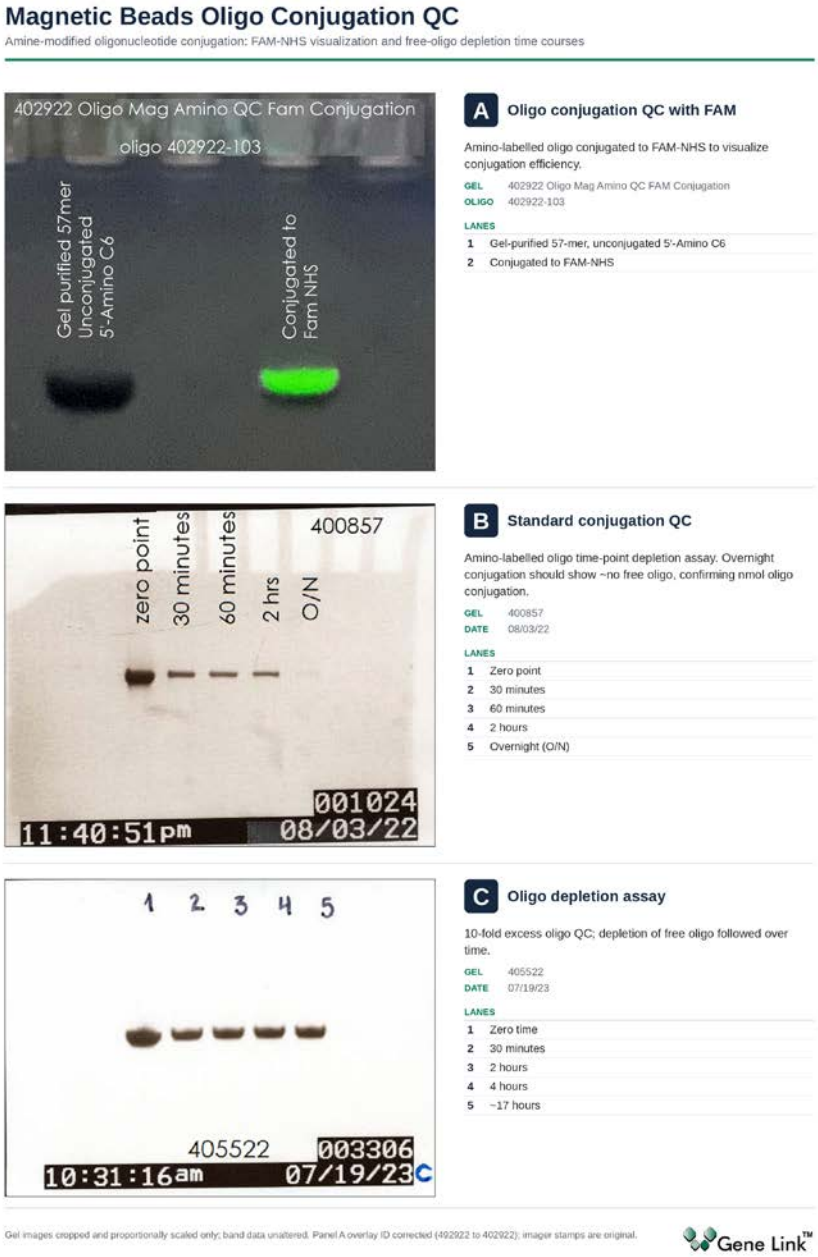
Application

1. Do not centrifuge the beads as it will collapse and form aggregates that will be difficult to re-suspend.
2. Use magnetic stand for separating the beads from the solution for all applications.
3. The material supplied is in 0.02% sodium azide (High Salt Buffer (0.5 M NaCl, 50 mM Tris-HCl pH 7.5 and 0.02% azide). Handle it with caution.
4. Handle it with caution. Before initial use, perform a few washes in the binding buffer of choice to equilibrate the new binding buffer and removal of azide.
5. For all hybridization/annealing the target must be denatured just as we do for PCR. Short protocol; heat denature target DNA/RNA at 95 degrees while maintaining separately the magnetic beads at 60 degrees in high salt buffer. Add your denatured target DNA/RNA while at 95 degrees to the magnetic beads. Let it cool to room temperature or by decreasing the temperature by 2 degrees every minute.
6. For DNA/oligo binding & release. Use High Salt (0.5 M NaCl, 50 mM Tris-HCl pH 7.5) for binding and washing, and Low Salt (50 mM Tris-HCl pH 7.5) or sterile water for elution. Elution at 60°C is preferable.
7. For RNA/oligo binding & release. Use High Salt (0.5 M NaCl, 50 mM Tris-HCl pH 7.5) for binding and washing. For RNA elution use RNase free water or preferably 1 mM Sodium Citrate pH 6.4. See this link. Elution at 60°C is preferable.

Quality Control & Oligo Conjugate Density

The binding capacity of oligos to the magnetic beads varies from batch to batch based on the NHS derivatization of the magnetic beads. An average NHS derivatization is ~1 nmols/ mg oligo thus considering 50-65% conjugation of the amino oligos to the beads is ~0.5 nmols/mg beads.

At Gene Link we do not calculate the exact concentration of oligo bound to the beads. Our quality control is based on oligo depletion and monitoring the depletion qualitatively by sampling time points at zero, 30 minutes, 1-2 hrs, 4 hrs and overnight (~17 hrs) and visualization by polyacrylamide gel electrophoresis (See photographs below). The oligo depletion assay is the depletion of oligo available free in solution as time progresses and the oligo conjugates to the solid phase performed by taking aliquots from the supernatant of the reaction mix.



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