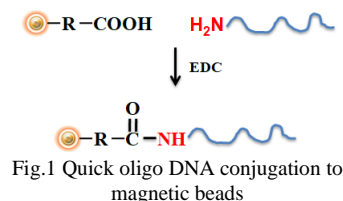


Quick Oligo-DNA Conjugation Kit

DNA has become an increasingly essential biomolecule in a variety of fields during the previous decade. DNA technology offers various applications, including forensic science, environmental investigations, diagnosis, and archeometry. In these fields, DNA microarrays and biosensors based on oligonucleotide DNA immobilization on solid substrates are utilized. The covalently immobilized oligonucleotide probe exhibits remarkable selectivity in subsequent hybridization operations with the complementary target and distinguishes itself from single-base mismatched oligonucleotide targets.

BcMag™ Quick Oligo-DNA Conjugation Kit is intended to quickly and efficiently immobilize oligonucleotide DNA to our proprietary magnetic beads. For a more secure attachment, the kit is designed to use cross-linker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to covalently immobilize amine-modified oligonucleotide to the surface of carboxyl-activated magnetic beads (Fig.1).



Features and Advantages

- Quick, Easy, and one-step protocol
- Neutral linkage—forms neutral amide bonds between carboxyls and amines.
- Stable covalent bond with minimal ligand leakage
- High immobilization efficiency
- Scalable -easily adjusts for sample size and automation
- Reproducible results

Specificities	
Composition	Carboxy-terminated magnetic beads
Bead Size	1µm diameter
Number of Beads	~1.7 x 10 ⁸ beads/mg
Magnetization	~45 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Stability	pH 4-10
Concentration	20 mg/ml in d ₂ H ₂ O
Binding Capacity	>10 µg Oligo-DNA (25 nucleotides)/mg
Storage	Store at 4°C upon receipt

Cat#	Kit components
CA-101	5ml Carboxy-terminated Magnetic beads.
	5ml 2x suspension Buffer:
	10ml 10x Washing Buffer
	0.15 g EDC(1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (Upon receipt store at -20°C)
CA-102	10ml Carboxy-terminated Magnetic beads.
	10ml 2x suspension Buffer:
	20ml 10x Washing Buffer
	0.3 g EDC(1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (Upon receipt store at -20°C)

PROTOCOLS

The protocol can be scaled appropriately up or down.

Materials Required

- Magnetic Rack (for manual operation)

Based on sample volume, the user can choose one of the following magnetic Racks: BcMag Rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag Rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag Rack-50 for holding one 50 ml



centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ Rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)

- Corning 430825 cell culture flask for large-scale purification (Cole-Parmer, Cat#EW-01936-22)
- Mini BlotBoy 3D Rocker, fixed speed, small 10" x 7.5" platform w/ flat mat (Benchmark Scientific, Inc. Cat# B3D1008) or compatible

A. Oligo-DNA preparation:

1. The oligo-DNA must be modified with an amino group at either 5' or 3' end (A commercial oligo synthesis company can provide such service).

Note: It is strongly recommended that 10-15 extra nucleotides be added to your oligo DNA sequence between the terminal amino group and your DNA sequence to overcome steric hindrance when you design your target oligo-DNA sequence. The oligo-DNA should be purified by standard desalting or other methods.

2. Resuspend the oligo-DNA in 1x suspension buffer at concentrations of 5-10 µg/ul (Optional: Aspirate 20 µl from the oligo solution, transfer to a new centrifuge tube, and label the tube as C1)

B. Coupling buffer preparation:

1. Prepare coupling buffer by adding 19 mg EDC to 1ml of 1x suspension buffer (Coupling buffer must be prepared fresh immediately before use)

C. Coupling

1. Shake the bottle to resuspend the BcMag Magnetic Beads thoroughly.
2. Transfer 1ml magnetic beads (20 mg/ml) to a tube. Place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack.
3. Remove the tube from the Rack and resuspend the beads thoroughly with 1ml suspension buffer. Place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack.
4. Repeat step 3 once.
5. Remove the tube from the Rack and resuspend the beads thoroughly with a 200µl coupling buffer. Mix the magnetic beads with 100-200 µg oligo-DNA prepared from Step A.2
6. Incubate the beads overnight at 50° C with continuous rotation.
7. Place the tube on a magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack (Optional: Aspirate 20 µl supernatant, transfer to a new centrifuge tube, and label the tube as C2).
8. Wash the beads three times with 1 ml of washing buffer at room temperature and twice with d_2H_2O at 65° C.
9. Resuspend the beads at 5 mg/ml in PBS buffer containing 0.2% NaN_3 and store them at 4° C.

Coupling efficiency calculation

1. Measure OD at A260

$$\text{Coupling Efficiency (\%)} = [(C1-C2)/C1] \times 100\%$$

C1: A260 Pre-Coupling oligo DNA Solution x dilution factor; C2: A260 post-Coupling oligo DNA Solution

2. Using fluorescent dye to quantify C1 and C2.

General references

1. Ferrier DC, Shaver MP, Hands PJW. Micro- and nano-structure-based oligonucleotide sensors. Biosens Bioelectron. 2015 Jun 15;68:798-810.
2. Sethi D, Gandhi RP, Kuma P, Gupta KC. Chemical strategies for immobilization of oligonucleotides. Biotechnol J. 2009 Nov;4(11):1513-29.
3. Zuo P, Ye BC. A novel immobilization strategy using oligonucleotide as linker for small molecule microarrays construction. Biosens Bioelectron. 2008 Jun 15;23(11):1694-700.



Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi & Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101