

Quick DNA-RNA Conjugation Kit

Many DNA microarray devices and chips have been created to detect DNA or RNA via solid phase hybridization. These biochips are generally based on the immobilization of DNA on a solid surface. They can collect and bind complementary DNA/RNA of interest from a tiny amount of samples. Immobilization of DNA/RNA on solid surfaces has grown in importance due to its applications in forensic science, environmental investigations, diagnosis and gene expression analysis, detection of single nucleotide polymorphisms and mutations, DNA sequencing or genetic disease diagnosis, gene expression analysis, detection of single nucleotide polymorphisms and mutations.

BcMag™ Quick DNA-RNA Conjugation Kit is intended to quickly and efficiently immobilize DNA, RNA, or phosphate-modified RNA/DNA oligos 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 5' phosphate group of DNA/RNA to amine-terminated magnetic beads (Fig.1). Our proprietary neutral coupling buffer makes the conjugation very efficient (>85%). The conjugated DNA is very stable and can be used for many downstream applications.

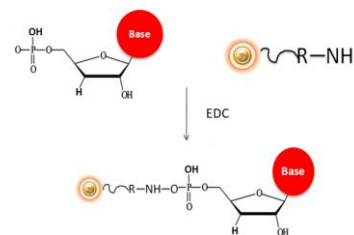


Fig.1 Quick immobilization of DNA/RNA to magnetic beads

Features and Advantages

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

| Specificities | |
|-----------------------|---|
| Composition | Amine-terminated magnetic beads |
| Bead Size | 2.5µm diameter |
| Number of Beads | ~ 1.68 x 10 ⁹ 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-103 | 100 mg | 2.5µm Amine-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-104 | 200 mg | 2.5µm Amine-terminated magnetic beads |
| | 10 ml | 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. DNA/RNA Sample Preparation

Note:

- All the samples (DNA/RNA) can not be suspended in TE or amine-containing buffer because it will reduce coupling efficiency.*
 - All the samples (DNA/RNA) must have a phosphate group at the 5' end. A commercial oligo synthesis company can provide such service or treat oligo-DNA with T4 DNA Kinase for oligo-DNA. The oligo-DNA should be purified by standard desalting or other methods.*
 - Amplified PCR products must be purified by standard procedure before coupling.*
 - < 1kb for all DNA/RNA samples is preferred for coupling.*
- All the samples (DNA/RNA) should be suspended in ultrapure water or 1x Suspension Buffer at a concentration of 5-10 µg/ul. (Optional: Aspirate 5-10 µl sample, transfer to a new centrifuge tube, and label the tube as C1)

B. Coupling buffer preparation

- 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

- Shake the bottle to resuspend the Magnetic Beads completely.
- 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.
- Remove the tube from the Rack and resuspend the beads thoroughly with a 1ml **Suspension Buffer**. Place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack.
- Repeat step 3 once.
- Remove the tube from the Rack and resuspend the beads thoroughly with a 200µl **Coupling Buffer**. Mix the magnetic beads with 100-200 µg DNA/RNA prepared from A1.
- Incubate the beads overnight at 50° C with continuous rotation.
- Place the tube on a magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack (Optional: Aspirate 5-10 µl supernatant, transfer to a new centrifuge tube and label the tube as C2).
- Wash the beads three times with 1 ml of **Washing Buffer** at room temperature and twice with ultrapure water at 65° C.
- Resuspend the beads at 5 mg/ml in PBS buffer containing 0.2% NaN₃ and store at 4° C.

D. Coupling efficiency calculation

- Measure OD at A260

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

C1: A260 Pre-Coupling DNA/RNA Solution x dilution factor; C2: A260 post-Coupling DNA/RNA Solution.

- Using fluorescent dye to quantify C1 and C2.



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 |