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Oligo $(dT)_{30}$ Magnetic Beads

1 Components

Component	HY-K0228-1 mL	HY-K0228-5 mL	HY-K0228-25 mL
Oligo $(dT)_{30}$ Magnetic Beads	1 mL	5 mL	25 mL

2 Introduction

MCE Oligo $(dT)_{30}$ Magnetic Beads are designed for the rapid isolation of highly purified, intact mRNA from eukaryotic total RNA or directly from crude extracts of cells, plant and animal tissues. The use of MCE Oligo $(dT)_{30}$ Magnetic Beads relies on base-pairing between the poly A tail of messenger RNA and the oligo dT sequences bound to the surface of the beads. The isolated mRNA can be used directly in most downstream applications in molecular biology: RT-PCR, solid-phase cDNA library construction, S1 nuclease analysis, ribonuclease protection assay, primer extension, dot and slot hybridization, in vitro translation experiments, RACE, subtractive hybridization, northern analysis, gene cloning, and gene expression analysis, etc.

3 Characteristics

Bead Concentration	10 mg/mL
Bead Mean Diameter	0.7 μm
Binding Capacity for mRNA	~10 μg mRNA/mg of beads
Density	1.4 g/cm^3
Storage Buffer	PBS pH 7.4, with 0.1% (v/v) ProClin 300

4 Protocol

Recommended Buffers and Solutions

Binding Buffer	20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 2 mM EDTA
Lysis/Binding Buffer	100 mM Tris-HCl, pH 7.5, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM dithiothreitol (DTT). If any precipitation is observed, warm the buffer to room temperature and shake until all the components are fully resuspended
Washing Buffer A	10 mM Tris-HCl, pH 7.5, 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS
Washing Buffer B	10 mM Tris-HCl, 0.15 M LiCl, 1 mM EDTA, pH 7.5

Preparation of Oligo $(dT)_{30}$ Magnetic Beads

Resuspend Oligo $(dT)_{30}$ Magnetic Beads thoroughly before use. Transfer 100 μL of beads from stock tube to the RNase-free 1.5 mL EP tube, add 1 mL of Binding Buffer and resuspend. Place the tube in Magnetic Stand for 2 minutes and remove the supernatant. Repeat 3-4 times. Resuspend the washed beads 1 mL of Binding Buffer.

Isolate mRNA from crude extracts of cells, plant and animal tissues (for reference)

1. Prepare Lysate

1.1 Plant and animal tissues

Grind frozen tissue in liquid nitrogen. Transfer the frozen powder to a new RNase-free EP tube. Add 1 mL of Lysis/Binding Buffer per 20-50 mg of animal tissue or 100 mg of plant tissue. Homogenize for 5 minutes until the tissue has completely lysed. Centrifuge at 14,000 rpm for 5 minutes, collect the supernatant. The lysate is now ready for mRNA isolation or can be frozen and stored at -80°C for Subsequent use.

Note: To reduce the viscosity of the solution, the genomic DNA can be sheared by blowing several times with a 1 mL syringe tip.

1.2 Cell Suspensions

Wash the cell suspension in phosphate-buffered saline (PBS) and centrifuge to obtain a cell pellet. Add 1.0 mL Lysis/Binding Buffer to the cell pellet (1-4 × 10^6 cells). Pipet up and down a couple of times to ensure the samples complete lysis. The lysate is now ready for mRNA isolation or can be frozen and stored at -80°C for subsequent use.

Note: To reduce the viscosity of the solution, the genomic DNA can be sheared by blowing several times with a 1 mL syringe tip.

2. Isolate mRNA from Crude Lysate

2.1 Remove the solution from the washed Oligo $(dT)_{30}$ Magnetic Beads and add the lysate. Mix beads and lysate. Allow binding by rotating on a mixer for 3-5 minutes at room temperature. Place the tube on the Magnetic Stand (HY-K0200) for 2 minutes and remove the supernatant.

2.2 Wash the beads twice at room temperature using the Magnetic Stand: Wash once with 1 mL Washing Buffer A, and once with 1 mL Washing Buffer B. Resuspend the beads thoroughly in the Washing Buffers to remove possible contaminants.

2.3 Perform one of the following:

a. If the bead-bound isolated mRNA is to be used in enzymatic downstream applications (e.g. solid-phase cDNA synthesis), wash one extra time with Washing Buffer B (500 µL) followed by one wash with the enzymatic buffer used in the downstream application.

b. To elute mRNA from the beads, remove the Washing Buffer B and add 10-20 µL 10 mM Tris-HCl. Incubate at 75-80°C for 2 minutes, then place the tube on the Magnetic Stand and quickly transfer the supernatant containing the mRNA to a new RNase-free tube. The final yield may vary somewhat between tissues/cells.

Purify mRNA from Total RNA (for reference)

Example of purifying 200 µg of total RNA

1. Adjust the volume of the 200 µg total RNA sample to 100 µL with distilled DEPC treated water or with 10 mM Tris-HCl pH 7.5. Add 100 µL of Binding Buffer.

2. Heat to 65°C for 2 minutes to disrupt secondary structures. Immediately place on ice.

3. Add the 200 µL of total RNA to the 100 µL washed beads. Mix thoroughly and allow binding by rotating continuously on a mixer for 5 minutes at room temperature.

Note: For every 200 µg total RNA, use 1 mg beads which are washed and resuspended in 100 µL of Binding Buffer.

4. Wash the beads twice in room temperature using the Magnetic Stand: Wash once with 1 mL Washing Buffer A, and once with 1 mL Washing Buffer B. Resuspend the beads thoroughly in the Washing Buffers to remove possible contaminants.

5. Perform one of the following:

a. If the bead-bound isolated mRNA is to be used in enzymatic downstream applications (e.g. solid-phase cDNA synthesis), wash one extra time with Washing Buffer B (500 µL) followed by one wash with the enzymatic buffer used in the downstream application.

b. To elute mRNA from the beads, remove the Washing Buffer B and add 10-20 µL 10 mM Tris-HCl. Incubate at 75-80°C for 2 minutes, then place the tube on the Magnetic Stand and quickly transfer the supernatant containing the mRNA to a new RNase-free EP tube. The final yield may vary somewhat between tissues/cells.

5 Storage

4°C, 3 years.

Do not freeze.

6 Precautions

1. Do not centrifuge, dry, freeze or exposure to a magnetic field for a long time.
2. All Buffers and consumables used for mRNA extraction should be RNase-free. RNase inhibitors may be added to the protocol at any steps. MCE RNase Inhibitor (HY-K1033) is recommended.
3. Beads/mRNA complex should be used immediately for RT-PCR. If storage is necessary, elute the mRNA from the beads and freeze.
4. LiDS is a strong inhibitor of enzymatic reactions. Thorough resuspension of beads/mRNA complex during washing and complete removal of washing buffer at each step will prevent carryover of LiDS and other salts to the downstream reaction.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.