Signosis
Innovative Plate Assay Solutions

Oligo-dT 96 mRNA Capture Kit

Catalog Number TC-0001 **(For Research Use Only)**

Introduction

The oligonucleotide 96 mRNA kit provides simple, cost-effective and rapid mRNA purification from suspension and adherent cells using oligonucleotide immobilization technology. The oligo-dT 96 mRNA kit can be used to carry out mRNA purification, cDNA synthesis, and PCR (CL-0002) or real-time PCR (CL-0003).

Principle

Oligonucleotide 96 mRNA kit is rapid to produce mRNA purification from a range of cell types, including suspension and adherent cells. In this technology, simply add cell lysate to the wells of a pre-coated 96 oligo-dT plate, and mRNA is then allowed to hybridize to the immobilized oligo-dT in each well. After washing the unbound, the isolated and purified mRNA can be further eluted for cDNA synthesis and PCR application.

Materials provided

- 96-well plate coated with oligo-dT (4^{o}C)
- Cell lysis buffer (4^{o}C)
- 10X Hybridization buffer (RM)
- 5xHybridization Wash buffer (RM)
- Elution Buffer (4^{o}C)
- Sealing films

Materials required but not provided

- Phosphate-buffered saline (PBS), pH 7.4
- RNase-free 96-well microplate

Assay Procedure

1. Hybridization on oligo dT plate

1) Prepare culture cells in a 96-well plate.
2) Remove the culture medium from each well.
3) Wash the wells using 200ul PBS per well. Remove the any trace of PBS in the well.
4) Add 100ul of Cell Lysis Buffer to each well.
5) Incubate at room temperature for at least 5 minutes. (Note: No shaking or mixing is necessary)
6) Transfer 100ul of cell lysate from each well to a well of pre-coated Oligo-dT plate and add 10ul 10x Hybridization buffer.
7) Incubate at room temperature for at least one hour.
   (Note: Incubation for 90 minutes on an orbital shaker (100rpm) will usually result in maximum mRNA yields.

2. Quantification of Hybridization

1) Remove the cell lysates from oligo-dT pre-coated plate.
2) Wash each well by adding 200ul Hybridization wash buffer.
3) Aspirate each well and wash by adding 200ul 1xHybridization wash buffer. Repeat the process three times for a total of four washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against a pile of clean paper towels.

3. Elution

1. Add 80ul Elution Buffer to each well of oligo-dT plate.
2. Cover the plate with a sealing film.
3. Incubate in 65^{o}C for 15 minutes.
4. After incubation, immediately transfer the mRNA elutes to a 96-well PCR microplate.
5. mRNA elutes can further be used for cDNA synthesis and PCR (CL-0002 or CL-0003) or stored at --80^{o}C for future use.