



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°C)
- Cell lysis buffer (4°C)
- 10X Hybridization buffer (RM)
- 5xHybridization Wash buffer (RM)
- Elution Buffer (4°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°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°C for future use

