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PRODUCT INFORMATION

mRNA Purification Kit from Total RNA

Product information for MT91928:

Kit contents

Components	MT91928, 25Preps
2x Binding Buffer	15ml
1x Binding Buffer	40ml
Wash Buffer	30 ml
RNA Elution	20 ml
Oligo(dT) Cellulose (5mg each)	125mg
LPA	200 µl
RNase-free ddH_2O	1ml
Protocol	1

Storage and stability
Transportation at 4^{o}C. RNA Elution and Oligo (dT) Cellulose store at -20^{o}C, others store at 4^{o}C. Valid for 1 year.

Reagents and Equipment to be Supplied by User
For mNRA Purification Kits:

- ü Water bath or heating block
- ü Sterile, RNase-free pipette tips, tubes
- ü Microcentrifuge capable of RCF12,000 *g*
- ü Isopropanol
- ü Disposable gloves

Introduction

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Eukaryotic mRNAs contain a stretch of “A” residues at their 3’end. The mRNA Purification Kit uses this characteristic to select mRNA from total RNA preparations. The total RNA used as starting material in this procedure can be prepared from any eukaryotic tissue or cultured cell. After just a single round of oligo(dT) selection, the poly(A) RNA will be essentially free of DNA and protein and sufficiently pure for virtually all uses, such as Northern blotting, RT-PCR, cDNA library construction in vitro translation.

Features

- **ü** mNRA has high purity and yield
- **ü** Easy to manipulate
- **ü** Compatible with many downstream applications including Northern blotting, RT-PCR, cDNA library construction, in vitro translation

Procedures

1. Add 0.5 ml 1 x Binding Buffer to 5mg Oligo(dT) Cellulose, mix thoroughly. Centrifuge at 2,000 *g* for 3 min at RT, then discard the filtrate from the tube.
2. Starting with 0.2~2 mg RNA in water or TE, add Nuclease-free water to adjust the volume to 0.5 ml. Add an equal volume 2X binding Buffer and mix thoroughly.
3. Add each RNA sample to 1 tube Oligo(dT) with gentle agitation. Centrifuge at 2,000 *g* for 3min at RT, then discard the filtrate from the tube.
4. Start to preheat RNA Elution at 65^{o}C, which could elute the poly(A)RNA from the Oligo(dT) Cellulose near the end of the procedure.
5. Add 0.5 ml 1X Binding Buffer to the Oligo(dT) Cellulose pellet, and mix thoroughly. Centrifuge at 2,000 *g* for 3 min at RT, and then discard the filtrate from the tube.
6. Repeat step 5 once
7. Add 0.5 ml Wash Buffer to the Oligo(dT) Cellulose pellet, and mix thoroughly. Centrifuge at 2,000 *g* for 3 min at RT, then discard the filtrate from the tube
8. Repeat steps 7 once.
9. Add 200 µl of prewarm (65^{o}C) RNA Elution to Oligo(dT) Cellulose. Close the tube and vortex briefly to thoroughly mix. Immediately centrifuge at 4,000 *g* for 3 min at RT. Collect the supernatant to a new tube.
10. Repeat step 9 once or twice
11. mRNA concentration
    a) Add an equal volume of isopropanol and 0.6% volume of LPA, mix thoroughly. Centrifuge at 12,000 g for 20 min at 4^{o}C, then carefully remove and discard the filtrate from the tube.
    b) Add 700 µl RNAse-Fee 70% alcohol, wash the mRNA once or twice, centrifuge at 12,000 g for 5 min at 4 degree Celsius, carefully remove and discard the filtrate from the tube
    c) Dissolve the mRNA pellet in 20-50 µl RNase-free ddH_2O.

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