mRNAdembeads Purification Maxi Kit (cat #06013)

Instruction manual for mRNA purification

Ademtech ⋆ Bioparc BioGalien ⋆ 27 Allée Charles Darwin
⋆ 33600 Pessac France
Tel : + 33 (0) 57 02 02 01, Fax : + 33 (0) 57 02 02 06,
E-mail : ademtech@ademtech.com

Version 1.4 Revised 03/14

I. Introduction

A. Overview
B. Description
C. Reagents provided with the kit and storage
D. Required Equipment to be supplied by the user
E. Total RNA Quantitation

II. mRNA Isolation protocol

A. Nucleo-Adembeads purification procedure
B. Protocol for mRNA isolation from Total RNA
C. Choose your own starting point

III. Quantitation: yield and quality of mRNA

IV. Troubleshooting

I. Introduction

A. Overview

A typical mammalian cell contains 10–30 pg total RNA. The majority of RNA molecules, however, are tRNAs and rRNAs. Depending on the cell type, tissue and metabolic state, mRNA accounts for 1–5% of the total cellular RNA.

Due to the low proportion of mRNA in the total cellular RNA pool, reducing the amount of rRNA and tRNA in a total RNA preparation increases the relative amount of mRNA. The mRNA enrichment is essential for construction of cDNA libraries and other applications where pure mRNA is highly desirable. The probability of selecting the right clone is greatly increased by reducing the amount of unwanted rRNA and tRNA. With pure, intact mRNA preparations, even low level messengers can easily be detected by *in vitro* translation, northern hybridization, Nuclease S1 protection analysis, expression-array and expression-chip analysis, or SAGE™ technology. Isolation of pure, intact mRNA is of great importance when characterizing mRNA species with these techniques.

The use of the kit relies on the base pairing between the poly A tail of the mRNA and the oligonucleotides (dT) sequence bound to the Nucleo-Adembeads.

Nucleo-Adembeads consists of superparamagnetic nanoparticles of uniform size (0,2 µm diameter) and a perfect spherical shape. Oligonucleotides $(dT)_{25}$ are linked to the surface of the superparamagnetic nanoparticles via Biotin-Streptavidin system. The true spherical shape eliminates non-specific binding associates with irregulary shape particles.

After processing the protocol using Nucleo-Adembeads, the mRNA is ready to use for downstream applications such as RT-PCR, Nothern Blotting, cDNA library construction, nuclease protection assay, *in vitro* translation, primer extension, subtractive cDNA cloning and reverse transcription.

B. Description

The mRNAdembeads Purification Maxi Kit is based on the use of superparamagnetic nanoparticles technology. This system eliminates the need of any type of column and does not require centrifugation step.

The superparamagnetic nanoparticles are coated with Streptavidin: Bio-Adembeads Streptavidin. The Biotin-Streptavidin interaction system enables a strong system to bind the biotinylated oligo (dT). The purified mRNA can be recovered in less than half an hour.

The isolated mRNA can be directly used in downstream applications in molecular biology such as: Northern Blot, RT-PCR, cDNA library construction, Nuclease S1 Protection Assay, *In vitro* Translation, Primer extension, Differential display.

RNA	Northern Blot	RT-PCR	cDNA library construction	Nuclease Protection Assay	In Vitro Translation	Primer extension	Differential display
RNA Total	++	++	+	++	-	++	+
Poly(A)+ RNA	+++	+++	+++	+++	+++	+++	+++

+++ recommended ++ satisfaisant + not recommended - impossible

Table 1: *Applications with mRNA and Total RNA*

The volume of each reagent needed for the purification procedure is detailed on page 4. The kit provides reagents for at least 50 isolations each from 100µg Total RNA.

B. Reagents provided with the kit and storage

The mRNAdembeads Purification Maxi Kit includes reagents for performing 10 mRNA isolations each from 1mg total RNA. This kit is provided to perform mRNA extraction from up to 10 mg Total RNA.

	Amount	Component	Storage
R1	4 tubes	Biotinylated Oligo$(dT)_{25}$	+ 4°C
R2	2 tubes x 1ml (5mg/ml)	Bio-Adembeads Streptavidin	+ 4°C
R3	2 tubes x 7.5 ml	Binding Buffer 1X	+ 4°C
R4	2 tubes x 2 ml	Binding Buffer 5X	+ 4°C
R5	2 tubes x 20 ml	Washing Buffer	+ 4°C
R6	2 tubes x 4 ml	Nuclease free Water	+ 4°C

Properly stored kits are guaranteed for 6 months from the date received. Note that the shipping is realized at room temperature which will not affect its stability. Do not freeze the particles, as this will decrease the system efficiency.

All the components of the kit have been prepared under ribonucleases free conditions and have been thoroughly tested to ensure optimal performance.

C. Required Equipment to be supplied by the user

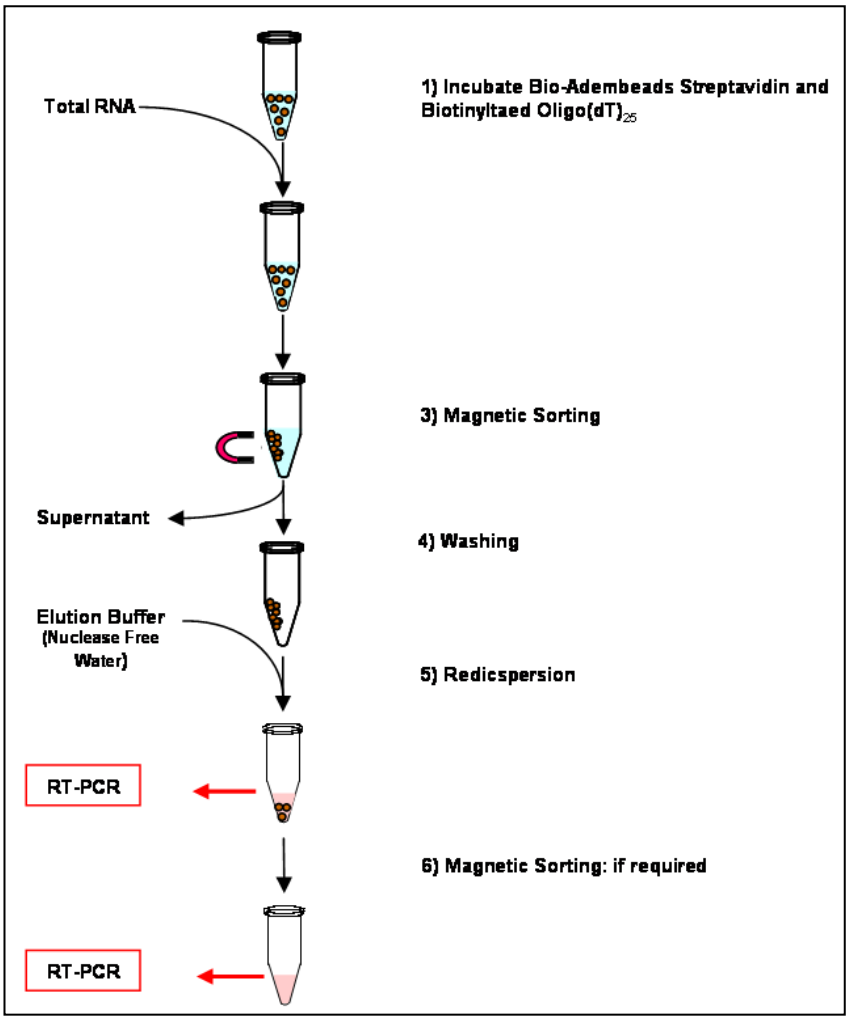
- Adem-Mag SV (#20101) or MODULO (#20105)
- Nuclease free microtubes
- RNase free tips
- Disposable gloves
- Spectrophotometer
- Shaker-incubator

D. Total RNA quantitation

Before using the Nucleo-Adembeads mRNA purification kit, Total RNA concentration of the starting material shoud be determined. The most used method to evaluate it is by measuring absorbance at 260nm. An absorbance of 1 unit at 260 nm corresponds to a RNA concentration of 40 µg / ml.
The ratio OD_{260}/OD_{280} provides an estimation of the purity of RNA with respect to contaminants that absorb in the UV, such as proteins. Pure RNA has an OD_{260}/OD_{280} ranging from 1.8 to 2 in 10 mM Tris·Cl, pH 7.5. The use of laboratory gloves is highly recommended at all step of the operating procedure in order to avoid nuclease contamination from skin.

II. mRNA Isolation from Total RNA

A. Nucleo-Adembeads purification procedure



B. Protocol for mRNA isolation from Total RNA

Total RNA should be resuspended in 720µl maximum.

B.1 Stock solution Preparation

This step is only required for the first utilisation of the kit

Biotinylated Oligo $(dT)_{25}$ working solution preparation: add 60µl of Nuclease Free Wate per 1 tube. Divide it into conveniently sized aliquots according to your utilization in order to avoid more than five freeze-thawing cycles.

 Aliquots of biotinylated oligo (dT)25 should be stored at -20°C.

Note: This procedure is described for purification of an amount from 600µg total RNA. (see Table 2)

B.2 Prepare Biotinylated Oligo$(dT)_{25}$ solution

1) Prepare the Biotinylated Oligo$(dT)_{25}$ solution by adding **173µl** Binding Buffer 1X to **12µl** of Biotinylated Oligo$(dT)_{25}$ working solution prepared and aliquoted in step B.1. In this case the final volume should be **185 µl**. (see Table 2)

B.3 Prepare Beads

1) Pipet **120µl (600µg) Bio-Adembeads Streptavidin** into a 1.5 ml microcentrifuge tube and place it on the Adem-Mag SV.
2) Pipett off the supernatant and wash the Bio-Adembeads Streptavidin with **600µl of Binding Buffer 1X** (See table 2).
3) Put the tube on the magnetic stand Adem-Mag SV and discard the supernatant after at least 1 minute.
4) Resuspend the Bio-Adembeads Streptavidin with the 185µl of Biotinylated Oligo (dT) solution and incubate under gentle rotation (500 rpm) for 5 minutes at room temperature.

B.4 Prepare Total RNA (This step can be performed at the same time as the step B.3)

Total RNA Solution Preparation:
Total RNA sample + Binding Buffer 5X + Nuclease Free Water qsp **900µl** (See table 2).
You have to obtain a final Binding Buffer concentration 1X.

Example: 600µg of total RNA at 2µg/µl
You need 480µl RNase Free Water and 120µl Binding Buffer 5X. (See Table 2)

1) Mix the Total RNA sample and the Nuclease Free water
2) Incubate at 70°C for 5 minutes and keep on ice at least 2 minutes.
3) Add the Binding Buffer 5X.

B.5 Bind to [Oligo (dT)25 - Bio-Adembeads Streptavidin] complex

1) Add the Total RNA solution to the [Oligo (dT) - Bio-Adembeads Streptavidin] complex and mix by pipetting.
2) Incubate under gentle rotation (500rpm) mixing for 10 minutes at room temperature.

B.6 Wash [Oligo (dT)25 - Bio-Adembeads Streptavidin] complex

1) Place the tubes on the magnet for at least 1 minute and remove the supernatant. Remove the tube from the magnet and wash with 600 µl Washing Buffer (see Table 2) and mix by pipetting.
2) Repeat twice step 1.

B.7 Elute mRNA

1) Remove the supernatant and resuspend Bio-Adembeads Streptavidin in desired amount of Nuclease free Water (50-200µl is recommended for this example) and mix by pipetting.
2) After 2 minutes at room temperature place the tube on the magnet during 5 minutes and transfer the supernatant (containing the eluted mRNA) into a new RNase free microtube.

C. Choose your own starting point

Total RNA (µg)	> 500 – 625	> 625 – 750	> 750 – 875	> 875 – 1000	
Biotinylated Oligo $(dT)_{25}$	use proportionnal quantity *(for 600 µg use 12µl)*				B.2
Final volume (µl) to prepare the Biotinylated Oligo$(dT)_{25}$	185	225	260	300	
Bio-Adembeads Streptavidin (µl)	= (Quantity in µg of Total RNA) / 5 *(for 600µg Total RNA use 120µl particles)*				B.3
Binding Buffer 1X (Washing Beads) (µl)	= Quantity in µl of Total RNA *(for 600µg Total RNA use 600µl Binding Buffer 1X)*				
Final volume (µl) of Total RNA Solution	900	900	900	900	B.4
Volume of Washing Buffer (µl)	= 3 x Quantity in µg of Total RNA *(for 600µg of Total RNA wash 3 times with 600µl of Washing Buffer)*				B.6
Elution Buffer (µl) (Nuclease free water)	Own choice				B.7

Table 2: Buffer amounts for mRNA isolation

III. Quantitation: yield and quality of mRNA

As described in the part I.G, the most common method for assessing the concentration and the purity of RNA is by reading the absorbance in a spectrophotometer at 260 nm and 280 nm.

Concentration:

An $OD_{260\ nm}$ corresponds approximately 40µg mRNA/ml.

Example: Volume of mRNA sample: 100µl
Dilute 5 µl of the sample in 195 µl DEPC water (dilution factor = 40)
Measured $OD_{260\ nm}$ = 0,5

mRNA concentration = 40µg /ml x $OD_{260\ nm}$ x dilution factor
= 40µg /ml x 0,5 x 40
= 800 µg /ml

Total amount = mRNA concentration x volume sample in ml
= 800 µg /ml x 0,1 ml
= 80 µg of mRNA

Purity:

The ratio OD_{260nm} / OD_{280nm} provides an estimation of the purity of the purified sample. The measured value should range from 1.8 to 2.1.

IV. Troubleshooting

A. Low yield of mRNA recovered

- **Be sure not to have a volume of Total RNA** higher than indicated in the table 2. If it is not possible precipitate RNA sample in order to have a higher concentration**.**
- **Be sure to use the corresponding amount** of Total RNA. Refer to the table 2, page 9.
- **The proportion of mRNA** in cells depend on the origin of cells and their state. 1 to 5% of mRNA is currently admitted.
- **Degraded mRNA**. It is possible to assess this information by put an aliquot of mRNA on a denaturing gel. To avoid any source of contamination we recommend wearing gloves during purification procedure. Be certain not to introduce any RNases during the procedure or later handling.

B. Impure RNA

- **Ribosomal RNA contamination.** The majority of RNA in any type of cells is rRNA (near 80%).

1) In the case of high rRNA amount, the purity can be improved by increasing the denaturation time (step B.4) to 10-15 minutes.
2) A second round of mRNA extraction could be performed to obtain a higher purity.

WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life.
Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price.
Claims for merchandise damaged in transit must be submitted to the carrier.