Instruction manual for 96 mRNA isolation system

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I. *Description*

mRNA purification procedure relies on the base pairing between the poly A tail of the mRNA and the biotinylated oligonucleotides $(dT)_{25}$ sequence bound to the Bio-Adembeads Streptavidin.

Nucleo-Adembeads consists of superparamagnetic nanoparticles of uniform size 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.

A. Reagents provided with the kit and storage

The automated mRNAdembeads Purification Kit includes reagents for performing 1x96 mRNA isolations each from up to 25µg Total RNA, 10^5 cells or 25µl whole blood.

	Components	**Volume**	**Storage**
R1	Bio-Adembeads Streptavidin (10mg/ml)	1ml (1%)	+ 4°C
R2	Biotinylated Oligo$(dT)_{25}$	Lyophilisate	+ 4°C
R3	Lysis Buffer	30ml	+ 4°C
R4	Binding Buffer 3X	15ml	+ 4°C
R5	Washing Buffer	40ml	+ 4°C
R6	Nuclease free Water	15ml	+ 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.

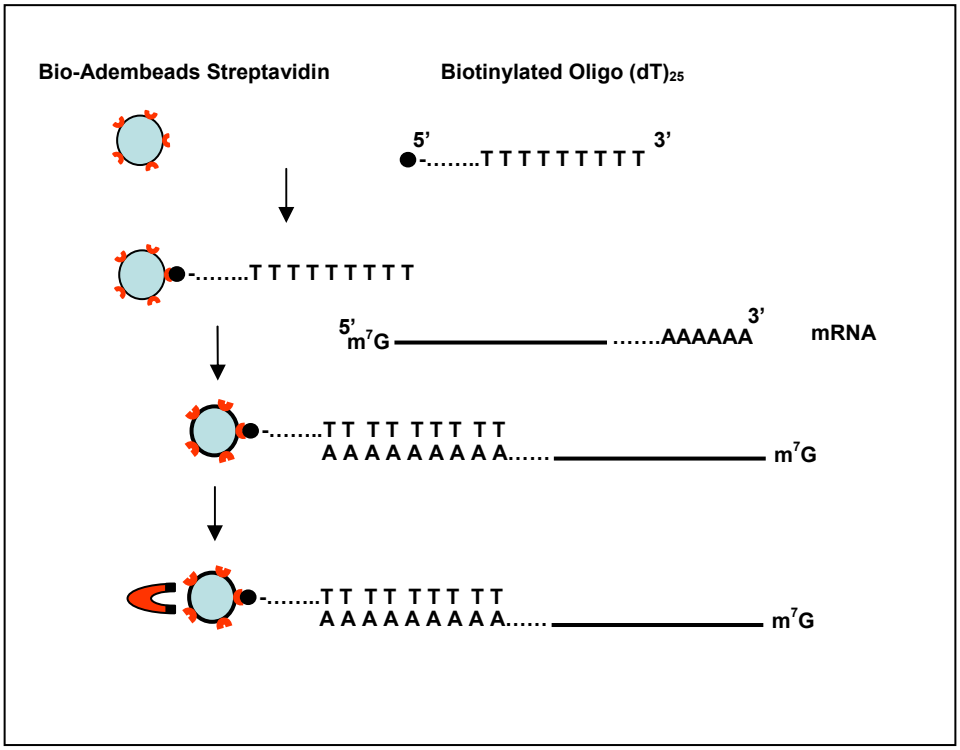
All the components of the kit have been prepared under ribonucleases free conditions and have been thoroughly tested to ensure optimal performance.

B. Required Equipment to be supplied by the user

- Magnetic separation device designed for 96-well plates
- Adapter for microplate
- 96-well U-bottom plates
- Rnase free tips
- Disposable gloves

II. mRNA Isolation protocol

A. Schematic procedure



B. Oligo $(dT)_{25}$ stock solution Preparation

Bio-Adembeads and Oligonucleotides $(dT)_{25}$ quantities, as well as reactional volumes are the same for each type of starting material: Total RNA, Cells lysate or whole blood.

Note: 1-2 minutes are recommended time for the magnetic separations.

Before starting:

Briefly centrifuge the tube and resuspend the lyophilisated **Oligo$(dT)_{25}$-Biotin** with 210µl of Nuclease Free Water. Once regenerated, the Oligo $(dT)_{25}$ must be stored at –20°C.

C. Protocol for mRNA isolation from Total RNA, Cell lysate or whole blood.

Reagents required per well:

	Total RNA (up to 25µg)	Cells number (up to 10^5 cells)	Whole Blood (up to 25µl)
Bio-Adembeads Streptavidin	**10µl**	**10µl**	**10µl**
Biotinylated Oligo $(dT)_{25}$	**2µl**	**2µl**	**2µl**
Binding Buffer 3X	**100µl + 48µl**	**100µl + 48µl**	**100µl + 48µl**
Lysis Buffer	**-**	**2 x 150µl**	**2 x 150µl**
Washing Buffer	**3 x 100µl**	**2 x 200µl**	**2 x 200µl**
Elution Buffer (Nuclease Free water)	50µl	50µl	50µl

B.1 Biotinylated Oligo $(dT)_{25}$ working solution preparation

The resuspended **Biotinylated Oligo $(dT)_{25}$** is diluted in the **Binding Buffer 3X** in order to have 2µl of Oligo $(dT)_{25}$ per 48µl of Binding Buffer 3X.

B.2 [Oligo $(dT)_{25}$ - Bio-Adembeads Streptavidin] complex preparation

1) **10µl (100µg) Bio-Adembeads Streptavidin** are dispensed into wells of the 96-well plate. Then add 100µl of **Binding Buffer 3X** to each well.
2) Put the plate on a magnetic stand and pipett off the supernatants.
3) Move off the plate and resuspend the Bio-Adembeads Streptavidin with **50µl of the Biotinylated Oligo $(dT)_{25}$/Binding Buffer 3X solution** (prepared in step B.1). Then, incubate for 5 minutes at room temperature.

B.3 Samples Preparation

B.3.1 Total RNA Preparation

1) Adjust the volume of Total RNA samples (up to 25µg) at 100µl with Nuclease Free Water (Elution Buffer).
2) Heat at 70°C for 5 minutes just before using.

B.3.2 Cell Lysate Preparation

1) Prepare cells in 50µl maximum of PBS or culture media
2) Add 150µl of Lysis Buffer and mix by pipetting.
3) Then heat at 70°C during 5 min. (optional)

B.3.3 Whole Blood Preparation

1) Add 150µl of Lysis Buffer and mix by pipetting.

Important: be sure to avoid heating the blood

B.4 mRNA capture by [Oligo $(dT)_{25}$ - Bio-Adembeads Streptavidin] complex

1) Add prepared samples solutions to the [Oligo $(dT)_{25}$ - Bio-Adembeads Streptavidin] complex and mix by pipetting.
2) Incubate for 10 minutes at room temperature.

B.5 Washing of the complex

Place the plate on the magnet, remove and discard the supernatants. Move off the plate and wash with:

- **100 µl Washing Buffer** for mRNA purification **starting from Total RNA**. Repeat twice this step.
- **150 µl Lysis Buffer** for mRNA purification **starting from cells or whole blood**. Repeat twice this step using 200µl of Washing Buffer.

Note: each volume is described into Table 1.

B.6 Elute mRNA

1) Place the plate on the magnet, remove and discard the supernatants.
2) Move off the plate and resuspend the complexes in desired volume of **Nuclease free Water** (mix by pipetting).
3) After 2 minutes at room temperature, place the plate on the magnet during 5 minutes and transfer **the supernatants (containing the mRNA)** into a collecting plate for storage.