Direct mRNAdembeads Purification Kit (cat #06021)

Instruction manual for Direct mRNA purification from

- **Cell Culture**
- **Blood**
- **Animal Tissues**
- **Plant Tissues**

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1. Overview

Biomagnetic separation technology is a simple technique based on the separation of superparamagnetic beads using a magnetic field. When added to a complex medium, the magnetic particles will bind to the target. This interaction is based on the specific affinity of the ligand to the surface of the beads. The resulting target-bead complex can be removed from the suspension using a magnet. The inherent benefits of magnetic handling allow for easy washing, separation and concentration of the target without any need of centrifugation or columns.

Superparamagnetic beads exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.

2. Product Principle

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 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 irregular 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.

Direct mRNAdembeads Purification kit

1. Product Description

1.1. General Description

The Direct mRNAdembeads Purification 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 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 ++ satisfying + not recommended - impossible

Table 1: *Applications with mRNA and Total RNA*

1.2. Kit capacities

The Direct mRNAdembeads purification kit offers flexibility for use with varying amount of starting material. The kit is not restricted to their nominal preparation size.

Sample	Amount of starting material	Number of isolation
Cells	10^6	30 isolations
Blood	200µl	30 isolations
Animal Tissues	25mg of liver	30 isolations
	50mg of brain	15 isolations
Plant tissues	25mg of embryonic wheat	15 isolations
	50mg of leaf	15 isolations
	1g of tomato fruit	15 isolations

Table 1: *Number of isolation per amount of starting material*

1.3. Reagents provided with the kit

The Direct mRNAdembeads Purification Kit includes reagents for performing 30 mRNA isolations each from 1 million of cells or 200µl of blood.

	Amount	Component	Storage
R1	0,6ml (10mg/ml)	Bio-Adembeads Streptavidin	+ 4°C
R2	Lyophilisate	Biotinylated Oligo(dT)$_{25}$	+ 4°C
R3	60ml	Lysis Buffer	+ 4°C
R4	4ml	Binding Buffer 1X	+ 4°C
R5	12ml	Washing Buffer	+ 4°C
R6	2ml	Nuclease free Water	+ 4°C

Table 2: *Reagent provided with the kit*

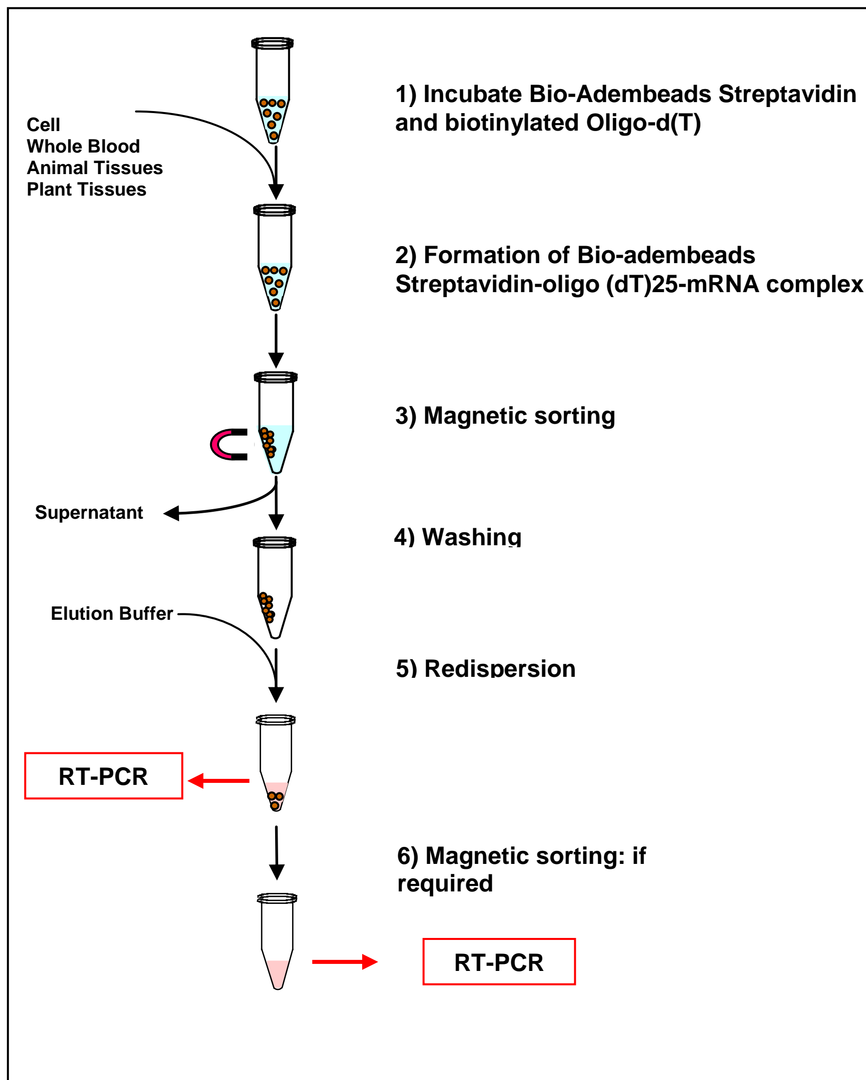
Properly stored Kits are guaranteed until the expiration date. Note that the shipping is realized at room temperature which will not affect its stability. Do not freeze the particles, as this will decrease the system efficiency. All the components of the kit have been prepared under ribonucleases free conditions and have been thoroughly tested to ensure optimal performance.

1.4. Required equipment to be supplied by the user

- Adem-Mag SV (#20101) or MODULO *Classic* (#20105):
- Auto-Mag (#30101):
- Nuclease free microtubes
- RNase free tips
- Disposable gloves
- Adapted Homogenizer
- Shaker-incubator

2. mRNA Isolation Protocol

2.1. Nucleo-Adembeads purification procedure



2.2. Protocol for mRNA isolation from cells and blood

Before starting the mRNA isolation procedure all buffers should be at room temperature to attain optimal performances.

2.2.1 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 Water. Divide it into conveniently sized aliquots according to your utilisation in order to avoid more than five freeze-thawing cycles.

Aliquots of biotinylated oligo $(\mathbf{dT})_{25}$ should be stored at -20°C.

Note: The following procedure is described for purification of an amount from 25µl to 100µl blood or 10^5 to $5x10^5$ cells. (see Table 2)

2.2.2 Prepare Biotinylated Oligo$(dT)_{25}$ solution

1) Prepare the Biotinylated Oligo$(dT)_{25}$ solution by adding **23,5µl Binding Buffer 1X** to **1,5µl** of Biotinylated Oligo$(dT)_{25}$ working solution prepared and aliquoted in step 2.2.1. In this case the final volume should be **25 µl** (see Table 2).

2.2.3. Prepare Beads

1) Pipet **15µl (150µg) Bio-Adembeads Streptavidin** into a 1.5 ml microcentrifuge tube and place it on the magnet.

2) Pipet off the supernatant and remove the test tube from the magnet. Then, wash the Bio-Adembeads Streptavidin with **100µl of Binding Buffer 1X** (See table 2).

3) Put the tube on the magnetic stand and discard the supernatant after at least 30 seconds.

4) Remove the test tube from the magnet and resuspend the Bio-Adembeads Streptavidin with the 25µl of Biotinylated Oligo $(dT)_{25}$ solution. Then, incubate under gentle rotation (500 rpm) for 5 minutes at room temperature.

2.2.4. Lysis step

1) <u>Cells</u>: Add 500µl of Lysis Buffer to the centrifuged cells and mix by pipetting. Homogenize the lysis sample by passing 5-6 times through a needle (23G, 0.6mm). Then, incubate at 70°C during 5 minutes just before using.

<u>Note:</u> if you are unable to obtain a visible pellet, directly add the Lysis Buffer to the cells (in a minimal volume of PBS or culture media).

2) <u>Blood</u>: Add directly 500µl of Lysis Buffer to the blood. **<u>Be careful to not heat the blood</u>**. Homogenize the blood lysis sample by passing 5-6 times through a needle (23G, 0.6mm).

2.2.5. Bind to [Oligo (dT)$_{25}$ – Bio-Adembeads Streptavidin] complex

1) Add the lysis 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.

2.2.6. Wash [Oligo(dT)$_{25}$ - Bio-Adembeads Streptavidin] complex

1) Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard the supernatant. Remove the tube from the magnet and add **500 µl Lysis Buffer** **(see Table 2)** and mix by pipetting.

2) Repeat twice step 1 using **200µl Washing Buffer.**

2.2.7. Elute mRNA

1) Remove the supernatant and resuspend Bio-Adembeads Streptavidin in desired amount of Nuclease Free Water (50µl 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.

2.2.8 Choose your own starting point

Whole Blood	≤ 25µl	25-100µl	100-200µl
Cells number	≤ 10^5	$10^5 – 5x10^5$	$5x10^5 – 10^6$

Biotinylated Oligo $(dT)_{25}$	1µl	1,5µl	2µl
Final volume to dilute the Biotinylated Oligo $(dT)_{25}$	25µl	25µl	25µl
Bio-Adembeads Streptavidin	10µl	15µl	20µl
Binding Buffer 1X	100µl	100µl	100µl
Lysis Buffer	250µl	500µl	1ml
1st Washing Step (use Lysis Buffer)	250µl	500µl	1ml
2nd and 3rd Washing Step (use Washing Buffer)	200µl	200µl	200µl
Elution Buffer	Own choice		

Table 2: Buffer amounts for mRNA isolation

2.2.9 To determine purity and mRNA concentration see Section 2.5

2.3. *Protocol for mRNA isolation from animal tissues*

Before starting the mRNA isolation procedure all buffers should be at room temperature to attain optimal performances.

2.3.1 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 Water. Divide it into conveniently sized aliquots according to your utilisation in order to avoid more than five freeze-thawing cycles.

Note:

- **This procedure is described for purification from 25mg of animal tissues. (see Table 3)**
- **Important: For some tissues, as liver or thymus, do not use more than 25mg of samples for the mRNA purification procedure.**

2.3.2 *Prepare Biotinylated Oligo$(dT)_{25}$ solution*

1) Prepare the Biotinylated Oligo$(dT)_{25}$ solution by adding **23µl** Binding Buffer 1X to **2µl** of Biotinylated Oligo$(dT)_{25}$ working solution prepared and aliquoted in step 2.3.1. In this case the final volume should be **25 µl** (see Table 3).

2.3.3 Prepare Beads

1) Pipet **20µl (200µg) Bio-Adembeads Streptavidin** into a 1.5 ml microcentrifuge tube and place it on the magnet.
2) Pipett off the supernatant and remove the test tube from the magnet. Then, wash the Bio-Adembeads Streptavidin with **100µl of Binding Buffer 1X** (See table 3).
3) Put the tube on the magnetic stand and discard the supernatant after at least 30 seconds.
4) Remove the test tube from the magnet and resuspend the Bio-Adembeads Streptavidin with the 25µl of Biotinylated Oligo $(dT)_{25}$ solution. Then, incubate under gentle rotation (500 rpm) for 5 minutes at room temperature.

From Animal Tissues

2.3.4 Lysis step

Efficient disruption and homogenization of the starting material is important. Complete disruption is absolutely required to release all the RNA contained in the sample. Homogenization reduces the viscosity of the lysates produced by disruption. The method used to disrupt tissue samples depends on the nature of the tissue.

Note : To avoid RNA degradation and to help to their homogenization, tissues can be kept frozen at -80°C or in liquid nitrogen.

1) Weight and homogenize **25mg** of tissue sample in an appropriated homogenizer in the presence of **1ml** Lysis Buffer

   Note :

   - **From fresh tissue**: Immediately after dissection, weigh tissue and add the Lysis Buffer.
   - **From frozen tissue:** Weigh frozen tissue and homogenize directly in Lysis Buffer.

2) Transfer the lysate in a microtube then centrifuge at 10000 x g for 5 min. Keep only the supernatant.

3) Homogenize the lysate by passing 5-6 times through a needle (23G, 0.6mm). Then, incubate at 70°C during 5 minutes just before using.

From Animal Tissues

2.3.5 Bind to [Oligo (dT)$_{25}$ – Bio-AdebBeads Streptavidin] complex

1) Add the lysis 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.

2.3.6 Wash [Oligo(dT)$_{25}$ - Bio-Adembeads Streptavidin] complex

1) Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard the supernatant. Remove the tube from the magnet and add **1ml Lysis Buffer (see Table 3)** and mix by pipetting.

2) Repeat twice step 1 using **200µl Washing Buffer.**

2.3.7 Elute mRNA

1) Remove the supernatant and resuspend Bio-Adembeads Streptavidin in desired amount of Nuclease Free Water (50µl 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.

2.3.8 *Choose your own starting point*

Tissues	**≤ 25mg**	25mg - 50mg
Biotinylated Oligo $(dT)_{25}$	**2µl**	4µl
Final volume to dilute the Biotinylated Oligo $(dT)_{25}$	**25µl**	25µl
Bio-Adembeads Streptavidin	**20µl**	40µl
Binding Buffer 1X	**100µl**	100µl
Lysis Buffer	**1ml**	1ml
1st Washing Step (use Lysis Buffer)	**1ml**	1ml
2st and 3rd Washing Step (use Washing Buffer)	**200µl**	200µl
Elution Buffer	**Own choice**	

Table 3: Buffer amounts for mRNA isolation from tissues

2.3.9 *To determine purity and mRNA concentration see Section 2.5.*

2.4. Protocol for mRNA isolation from plant tissues

Before starting the mRNA isolation procedure all buffers should be at room temperature to attain optimal performances.

2.4.1 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 Water. Divide it into conveniently sized aliquots according to your utilisation in order to avoid more than five freeze-thawing cycles.

Note: The following procedure is described for purification from 25mg of embryonic wheat tissues (see Table 4).

2.4.2 *Prepare Biotinylated Oligo(dT)$_{25}$ solution*

1) Prepare the Biotinylated Oligo(dT)$_{25}$ solution by adding **21µl** Binding Buffer 1X to **4µl** of Biotinylated Oligo(dT)$_{25}$ working solution prepared and aliquoted in step 2.4.1. In this case the final volume should be **25 µl** (see Table 4).

2.4.3. Prepare Beads

1) Pipet **40µl (400µg) Bio-Adembeads Streptavidin** into a 1.5 ml microcentrifuge tube and place it on the magnet.
2) Pipett off the supernatant and remove the test tube from the magnet. Then, wash the Bio-Adembeads Streptavidin with **100µl of Binding Buffer 1X** (See table 4).
3) Put the tube on the magnetic stand and discard the supernatant after at least 30 seconds.
4) Remove the test tube from the magnet and resuspend the Bio-Adembeads Streptavidin with the 25µl of Biotinylated Oligo (dT)$_{25}$ solution. Then, incubate under gentle rotation (500 rpm) for 5 minutes at room temperature.

2.4.4 Lysis step

Tissue	Suggested method
Freshly dissected hard tissue (roots, leaf)	Freeze and grind in liquid N_2 and use manual or electric homogenizer
Freshly dissected and frozen tissue (soft to medium)	Use manual or electric homogenizer
Freshly and water rich tissue (tomato, berries)	Centrifuge 5 min 10000xg to eliminate the juice and use manual or electric homogenizer

1) Weight and homogenize **25mg** of tissue sample in an appropriated homogenizer in the presence of **1ml** Lysis Buffer
2) Transfer the lysis sample in a microtube and centrifuge at 10000g for 5 min. Keep the supernatant.
3) Finally, pass the lysis sample 5-6 times through a needle (23G, 0.6mm) if necessary and incubate at 70°C during 5 minutes just before using.

2.4.5 Bind to [Oligo $(dT)_{25}$ – Bio-Adembeads Streptavidin] complex

1) Add the lysis 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.

2.4.6 Wash [Oligo$(dT)_{25}$ - Bio-Adembeads Streptavidin] complex

1) Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard the supernatant. Remove the tube from the magnet and add with **1ml Lysis Buffer** (see Table 4) and mix by pipetting.
2) Repeat twice step 1 using **200µl Washing Buffer.**

2.4.7 Elute mRNA

1) Remove the supernatant and resuspend Bio-Adembeads Streptavidin in desired amount of Nuclease Free Water (50µl 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.

2.4.8. Choose your own starting point

Tissues	**Embryonic Wheat**		**Mature fruits (Tomato or grappe)**	**Leaf**
	≤ 10mg	10mg - 25mg	1g	50mg
Biotinylated Oligo $(dT)_{25}$	**2µl**	4µl	4µl	4µl
Final volume to dilute the Biotinylated Oligo $(dT)_{25}$	**25µl**	25µl	25µl	25µl
Bio-Adembeads Streptavidin	**20µl**	40µl	40µl	40µl
Binding Buffer 1X	**100µl**	100µl	100µl	100µl
Lysis Buffer	**1ml**	1ml	1ml	1ml
1st Washing Step (use Lysis Buffer)	**1ml**	1ml	1ml	1ml
2st and 3rd Washing Step (use Washing Buffer)	**200µl**	200µl	200µl	200µl
Elution Buffer	**Own choice**			

Table 4: Buffer amounts for mRNA isolation from various plant tissues

2.4.9 To determine purity and mRNA concentration see Section 2.5.

From Plant Tissues

2.5 Quantification: yield and quality of mRNA

The most common method for assessing the concentration and the purity of RNA is by reading the absorbance in a spectrophotometer at 260 nm, 280 nm and 230nm.

- **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 RNAse Free 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.

The ratio OD_{260nm} / OD_{230nm} provides an estimation of the purity as against polysaccharides and polyphenol (important for somes plants). The measured value should range around 2.0 and upper.

2.6 Eliminating genomic DNA contamination

Generally, DNAse digestion is not required since Nucleo-Adembeads Technology efficiency removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for RNA applications that are sensitive to very small amounts of DNA. It is possible to use DNase I (ribonuclease and protease free) at two separated mRNA extraction protocol:

1- DNA can removed by a DNase digestion during wash step.

Lysis of the samples and binding of mRNA to the beads are performed according to the standard protocols. Instead of performing [Oligo(dT)$_{25}$ - Bio-Adembeads Streptavidin] complex wash step (exp. 2.2.6, 2.3.6, 2.4.6) follows steps 1-8.

1) Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard the supernatant. Remove the tube from the magnet and add 500µl or 1ml Lysis Buffer (see Table 2, 3 or 4) according the starting samples. Mix by pipetting.
2) Repeat step 1 using 200µl Washing Buffer.
3) Add 20µl of 10X Activation Buffer for DNase
4) Add 5µl of DNase I and mix by pipetting.
5) Incubate 10 minutes at Room Temperature.
6) Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard the supernatant.
7) Remove the tube from the magnet and add 200µl Washing Buffer and mix by pipetting.
8) Place the tube on the magnet for at least 1 minute or until supernatant clearing.
9) To elute mRNA see section 2.4.7

2- DNA can also removed by a DNase digestion following RNA purification.

Lysis of the samples and binding of mRNA to the beads and washing are performed according to the standard protocols. Instead of performing mRNA elution step (exp.2.2.7, 2.3.7, 2.4.7) follows steps 1-6.

1. Remove the supernatant and resuspend Bio-Adembeads Streptavidin in desired amount of Nuclease Free Water (50µl 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.
3. Add 5µl of DNase I and mix by pipetting.
4. Incubate 10 minutes at Room Temperature.
5. Add 5µl of 10X Stop Buffer (for 50µl of Nuclease Free Water).
6. Incubate at 65°C for 10 minutes to inactivate the DNase.

Treated RNA sample is ready to be used in downstream applications as RT-PCR.

Troubleshooting

A. Low yield of mRNA recovered

- **Be sure to not have a quantity of starting material** higher than indicated in the tables 2, 3, 4.

- **The proportion of mRNA** in cells and tissues could depend on their origin and their state. 1 to 5% of mRNA is currently admitted.

- **Degraded mRNA**. To avoid any source of contamination we recommend wearing gloves during purification procedure. Be sure 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 to 10 min the incubation time at 70°C at Lysis steps.
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.