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**ademtech**

## **Direct mRNA dembeads Purification Kit (cat #06021)**

**Instruction manual for  
Direct mRNA purification from**

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



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## General Overview

### 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)<sub>25</sub> are linked to the surface of the superparamagnetic nanoparticles via Biotin-Streptavidin system. The true spherical shape eliminates non-specific binding associates with irregularly shape particles. After processing the protocol using Nucleo-Adembeads, the mRNA is ready to use for downstream applications such as RT-PCR, Northern Blotting, cDNA library construction, nuclease protection assay, *in vitro* translation, primer extension, subtractive cDNA cloning and reverse transcription.

## Direct mRNA dembeads Purification kit

### 1. Product Description

#### 1.1. General Description

The Direct mRNA dembeads 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      ++ satisfaisant    + not recommended    - impossible

**Table 1:** Applications with mRNA and Total RNA

## 1.2. Kit capacities

The Direct mRNA dembeads 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 <sup>6</sup>             | 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 mRNA dembeads 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<br>(10mg/ml) | Bio-Adembeads<br>Streptavidin        | + 4°C   |
| R2 | Lyophilisate       | Biotinylated Oligo(dT) <sub>25</sub> | + 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

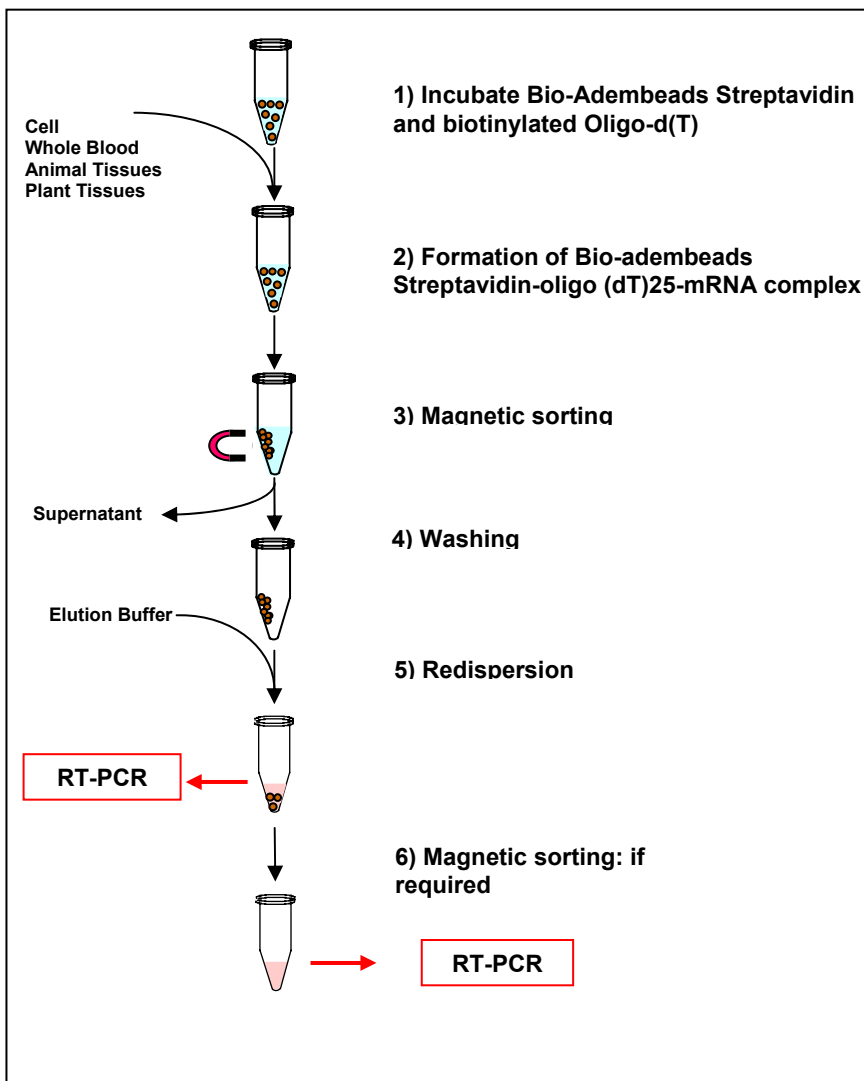
Properly stored Kits are guaranteed until the expiration date. Note that the shipping is realized at room temperature which will not affect its stability. Do not freeze the particles, as this will decrease the system efficiency. All the components of the kit have been prepared under ribonucleases free 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 MSV (#20104):
- 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)<sub>25</sub> working solution preparation:** add 60µl of Nuclease Free Water. Divide it into conveniently sized aliquots according to your utilization in order to avoid more than five freeze-thawing cycles.

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

### 2.2.2 Prepare Biotinylated Oligo(dT)<sub>25</sub> solution

- 1) Prepare the Biotinylated Oligo(dT)<sub>25</sub> solution by adding **23,5µl Binding Buffer 1X** to **1,5µl** of Biotinylated Oligo(dT)<sub>25</sub> 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) 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 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)<sub>25</sub> solution. Then, incubate under gentle rotation (500 rpm) for 5 minutes at room temperature.



#### 2.2.4. Lysis step

- 1) Cells: 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.

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

#### 2.2.5. Bind to [Oligo (dT)<sub>25</sub> – 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)<sub>25</sub> - 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  | $\leq 25\mu\text{l}$ | <b>25-100<math>\mu\text{l}</math></b>    | 100-200 $\mu\text{l}$  |
| Cells number | $\leq 10^5$          | <b><math>10^5 - 5 \times 10^5</math></b> | $5 \times 10^5 - 10^6$ |

|   |                   |                                    |                   |
|---|-------------------|------------------------------------|-------------------|
| <b>Biotinylated Oligo (dT)<sub>25</sub></b>                           | 1 $\mu\text{l}$   | <b>1,5<math>\mu\text{l}</math></b> | 2 $\mu\text{l}$   |
| Final volume to dilute the Biotinylated Oligo (dT) <sub>25</sub>      | 25 $\mu\text{l}$  | <b>25<math>\mu\text{l}</math></b>  | 25 $\mu\text{l}$  |
| <b>Bio-Adembeads Streptavidin</b>                                     | 10 $\mu\text{l}$  | <b>15<math>\mu\text{l}</math></b>  | 20 $\mu\text{l}$  |
| Binding Buffer 1X   | 100 $\mu\text{l}$ | <b>100<math>\mu\text{l}</math></b> | 100 $\mu\text{l}$ |
| Lysis Buffer  | 250 $\mu\text{l}$ | <b>500<math>\mu\text{l}</math></b> | 1ml               |
| 1 <sup>st</sup> Washing Step (use Lysis Buffer)                       | 250 $\mu\text{l}$ | <b>500<math>\mu\text{l}</math></b> | 1ml               |
| 2 <sup>st</sup> and 3 <sup>rd</sup> Washing Step (use Washing Buffer) | 200 $\mu\text{l}$ | <b>200<math>\mu\text{l}</math></b> | 200 $\mu\text{l}$ |
| 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)<sub>25</sub> working solution preparation:** add 60µl of Nuclease Free Water. Divide it into conveniently sized aliquots according to your utilization 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)<sub>25</sub> solution

- 1) Prepare the Biotinylated Oligo(dT)<sub>25</sub> solution by adding **23µl** Binding Buffer 1X to **2µl** of Biotinylated Oligo(dT)<sub>25</sub> 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)<sub>25</sub> solution. Then, incubate under gentle rotation (500 rpm) for 5 minutes at room temperature.

### 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) Weigh 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.

### 2.3.5 Bind to [Oligo (dT)<sub>25</sub> – 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.

### 2.3.6 Wash [Oligo(dT)<sub>25</sub> - 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) <sub>25</sub>                                 | 2µl        | 4µl   |
| Final volume to dilute the Biotinylated Oligo (dT) <sub>25</sub>      | 25µl       | 25µl  |
| Bio-Adembeads Streptavidin  | 20µl       | 40µl  |
| Binding Buffer 1X   | 100µl      | 100µl |
| Lysis Buffer  | 1ml        | 1ml   |
| 1 <sup>st</sup> Washing Step (use Lysis Buffer)                       | 1ml        | 1ml   |
| 2 <sup>st</sup> and 3 <sup>rd</sup> 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)<sub>25</sub> working solution preparation:** add 60µl of Nuclease Free Water. Divide it into conveniently sized aliquots according to your utilization 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)<sub>25</sub> solution

- 1) Prepare the Biotinylated Oligo(dT)<sub>25</sub> solution by adding **21µl** Binding Buffer 1X to **4µl** of Biotinylated Oligo(dT)<sub>25</sub> 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)<sub>25</sub> 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 <sub>2</sub> 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)<sub>25</sub> – 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)<sub>25</sub> - 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<br>(Tomato or<br>grappe)<br>1g | Leaf<br>50mg |
|---------|-----------------|-------------|--|--------------|
|         | ≤ 10mg          | 10mg - 25mg |  |              |

|   |            |       |       |       |
|---|------------|-------|-------|-------|
| Biotinylated Oligo (dT) <sub>25</sub>                                 | 2µl        | 4µl   | 4µl   | 4µl   |
| Final volume to dilute the Biotinylated Oligo (dT) <sub>25</sub>      | 25µl       | 25µl  | 25µl  | 25µl  |
| Bio-Adembeads Streptavidin  | 20µl       | 40µl  | 40µl  | 40µl  |
| Binding Buffer 1X   | 100µl      | 100µl | 100µl | 100µl |
| Lysis Buffer  | 1ml        | 1ml   | 1ml   | 1ml   |
| 1 <sup>st</sup> Washing Step (use Lysis Buffer)                       | 1ml        | 1ml   | 1ml   | 1ml   |
| 2 <sup>st</sup> and 3 <sup>rd</sup> 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.



## 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  $OD_{260\text{ nm}}$  corresponds approximately  $40\mu\text{g mRNA/ml}$ .

Example: Volume of mRNA sample:  $100\mu\text{l}$

Dilute  $5\mu\text{l}$  of the sample in  $195\mu\text{l}$  RNase Free water (dilution factor = 40)

Measured  $OD_{260\text{ nm}} = 0,5$

$$\begin{aligned}\text{mRNA concentration} &= 40\mu\text{g /ml} \times OD_{260\text{ nm}} \times \text{dilution factor} \\ &= 40\mu\text{g /ml} \times 0,5 \times 40 \\ &= 800\mu\text{g /ml}\end{aligned}$$

$$\begin{aligned}\text{Total amount} &= \text{mRNA concentration} \times \text{volume sample in ml} \\ &= 800\mu\text{g /ml} \times 0,1\text{ ml} \\ &= 80\mu\text{g of mRNA}\end{aligned}$$

### ■ Purity:

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

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

## 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.