Miltenyi Biotec

µMACS™ mRNA Isolation Kits

Order no. 130-130-152
130-122-751

Miltenyi Biotec

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1. Description

1.1 Components and size

▲ All buffers and MACS® Columns included in the µMACS™ mRNA Isolation Kits are evaluated for the absence of RNase activity.

µMACS mRNA Isolation Kit	Small Scale	Large Scale
Order no.	130-122-751	130-130-152
Reactions	20	8
Oligo (dT) MicroBeads	1 mL	2× 1 mL
Lysis/Binding Buffer	40 mL	60 mL
Wash Buffer	20 mL	20 mL
Elution Buffer	2× 1.3 mL	2× 1.3 mL
No. of columns	20 µ Columns	8 M Columns
No. of LysateClear Columns	20	8
No. of centrifugation tubes	20	8

Components

Oligo(dT) MicroBeads: non-sedimenting MicroBeads conjugated to oligo $(dT)_{25}$.

Lysis/Binding Buffer

Wash Buffer

Elution Buffer

Small Scale LysateClear Columns (maximal reservoir volume: 1 mL; capacity: lysate from a

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maximum of 1×10^7 cells, 30 mg animal tissue, or 100 mg plant tissue) and **20 centrifugation tubes**.

Large Scale LysateClear Columns (maximal reservoir volume: 5 mL; capacity: lysate from a maximum of 5×10^7 cells, 150 mg animal tissue, or 500 mg plant tissue) and **8 centrifugation tubes**.

µ Columns (capacity: up to 10 µg mRNA).

M Columns (capacity: up to 50 µg mRNA).

▲ MACS µ Columns or M Columns cannot be used for cell separations.

Product format Oligo(dT) MicroBeads are supplied in a suspension containing 0.1% SDS.
Lysis/Binding Buffer is a high salt buffer containing 1% SDS.
Wash Buffer is a low salt buffer.
Elution Buffer is RNase-free H_2O.

Storage Store Buffer Set Box containing buffers and MicroBeads protected from light at 2–8 °C. Do not freeze. The expiration dates are indicated on the box labels. Store MACS µ Columns and M Columns as well as LysateClear Columns dry at 10–35 °C, protected from light.

1.2 mRNA isolation with MACS® Technology

Eukaryotic messenger RNA (mRNA), the transcript for protein synthesis, is the basis for information about specific gene expression profiles in cells and tissue. While mRNA represents only 1–5% of the total RNA, many downstream applications such as RT-PCR, microarray analysis, Northern blotting, or cDNA synthesis are performed to analyze mRNA expression. However, accurate gene expression analyses depend on mRNA isolation methods that circumvent common pitfalls: DNA contaminations and degradation of the RNA during the isolation can lead to false results, contaminating ribosomal RNA (rRNA) lowers the efficiency of the reverse transcription, and mRNA is often lost during conventional precipitation and washing steps.

The µMACS mRNA Isolation is a robust and reproducible procedure based on MACS Technology. It enables direct isolation of mRNA without prior preparation of total RNA. With the µMACS mRNA Isolation Kits full length, intact mRNA can be obtained from fresh, frozen, or cultured cells[1], animal[2, 3] or plant tissue, whole blood, or total RNA[4]. The core components are superparamagnetic Oligo(dT) MicroBeads that hybridize to the stretch of adenosine residues at the 3′end of eukaryotic mRNA. The magnetically labeled mRNA can then be isolated on µ Columns or M Columns placed in a MACS Separator. After magnetic isolation, the mRNA can be eluted from the column in a small volume ready for downstream analysis (refer to figure 2).

The fast mRNA preparation in 15–30 minutes directly from cells or tissue reduces the risk of RNA degradation, while the Column Technology provides effective washing steps to minimize DNA or RNA contamination. In addition, µMACS RNA Isolation is a very sensitive method enabling detection of rare transcripts and gene expression analysis from small biological samples.

For subsequent cDNA synthesis, a reverse transcription can be performed in the µ Column. The µMACS One-step cDNA Kit combines direct mRNA isolation with in-column cDNA synthesis. Thereby, loss of material is reduced compared to other methods which require extra pipetting and purification steps of in-tube reactions. This is especially important when working with small sample amounts for sensitive analyses.

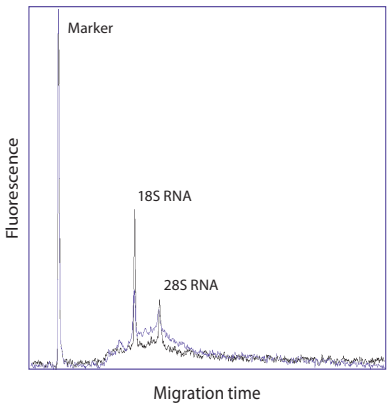


Figure 1: Elution diagramm of mRNA
mRNA was isolated either with µMACS Technology (purple) or latex resin (black) from 200 µg total RNA of mouse spleen (Agilent Bioanalyzer 2100, Agilent Technologies). The elevated fluorescence surrounding the 18S and 28S RNA peaks reflects the amount of the eluted mRNAs. The height of the ribosomal RNA peaks visualizes the amount of 18S and 28S RNA in the eluates.

Principle and time schedule for µMACS mRNA Isolation Kits

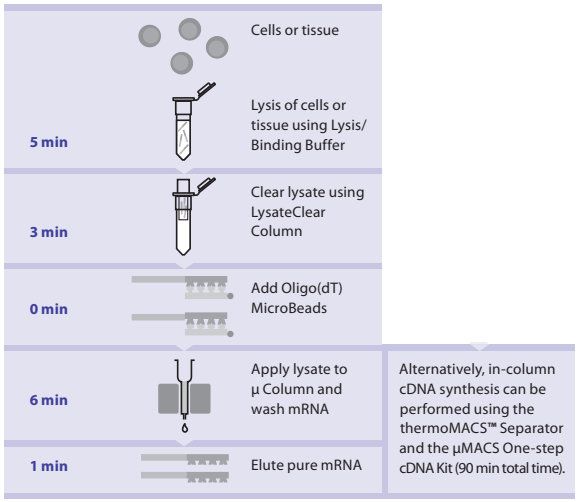


Figure 2: Isolation of mRNA with Oligo(dT) MicroBeads

1.3 Kit capacities

µMACS mRNA Isolation Kit – Small Scale (µ Columns)

- This kit is for isolation of mRNA from a maximum of 1×10^7 cells, 30 mg human or animal tissue, 100 mg plant tissue, or 200 µg total RNA. Cell number of 1×10^7 cells, or 30 mg human or animal tissue, typically yield 1–10 µg mRNA, depending on transcription activity. Some resting cells, e.g. lymphocytes, may contain significantly lower amounts of mRNA. mRNA yields from 1×10^7 primary cells isolated with MACS Technology such as $CD19^+$, $CD133^+$ and BDCA-4^+ cells were 0.5–1µg. Using 30 mg mouse tissue, mRNA yields were 1–2 µg for brain/heart/lung/small intestine, approximately 3 µg for spleen/ kidney and about 6 µg for liver.
- Isolation of mRNA from up to 0.5 mL of human whole blood.
- Isolation of mRNA from up to 2×10^8 yeast protoplasts (please refer to the special protocol "mRNA isolation from yeast cells", www.miltenyibiotec.com).

µMACS mRNA Isolation Kit – Large Scale (M Columns)

- Isolation of mRNA from a maximum of 5×10^7 cells, 150 mg human or animal tissue, or 500 mg plant tissue.
- Isolation of mRNA from up to 2.5 mL human whole blood.

1.4 Reagent and instrument requirements

▲ All additionally required equipment must be RNase-free.

- For homogenization and lysis of tissue: Mortar and pestle or rotor-stator homogenizer.
- For mechanical shearing of DNA to homogenize tissue, whole blood, and cells: Sterile, RNase-free 21G needles and 1–5 mL syringes.
- RNase-free tubes and pipette tips.
- **MACS Separator**

  For small scale isolation in µ Columns:

  µMACS Separator or thermoMACS™ Separator.

  For large scale isolation in M Columns:

  MiniMACS™ Separator, OctoMACS™ Separator for simultaneous processing of up to 8 samples, or SuperMACS™ II Separator plus appropriate adapter.
- Heating block or water bath pre-warmed to 70 °C.
- Centrifuge suitable for 2 mL tubes (Small Scale) or 15 mL tubes (Large Scale) with a centrifuging capacity of ≥13,000×g or ≥5,000×g, respectively.
- (Optional) Antifoam A reagent (1%), Sigma-Aldrich®, can be added to prevent extensive foam formation during sample homogenization.
- (Optional) RNA stabilizing buffer.

1.5 Related products

- µMACS One-step cDNA Kit (# 130-091-902)
- µMACS One-step cDNA Starting Kit (# 130-091-989)
- For all MACS Products for cell separation, please visit www.miltenyibiotec.com/cellseparation

2. Protocols for mRNA isolation

▲ All additionally required equipment must be RNase-free.

▲ Thorough sample homogenization and cell lysis, as well as the reduction of viscosity of lysates, are very important (please refer to chapter 3, Tips & hints).

2.1 Sample preparation

µMACS mRNA isolation is compatible with the following sample types: Adherent or suspension cell samples (section 2.1.1); human, animal, or plant tissue (2.1.2), and whole blood (2.1.3). Also, it is used for isolation of mRNA from total RNA (2.1.4).

2.1.1 Adherent or suspension cells

Suspension cells are lysed immediately after harvesting (section 2.1.1.1). Adherent cells can be lysed directly in the cell culture vessel (section 2.1.1.2), or detached with trypsin or EDTA before lysis (section 2.1.1.3).

Small Scale **preparation in µ Columns:** Use up to 1×10^7 cells.
Large Scale preparations in M Columns: Use up to 5×10^7 cells.

Before starting

▲ Warm Elution Buffer to 70 °C using a heating block or water bath. Adjust Lysis/Binding Buffer and Wash Buffer to room temperature.

▲ To achieve a high yield of mRNA, place the heating block close to the Separator and work fast to avoid cooling of the Elution Buffer before applying it to the column.

2.1.1.1 Lysis of suspension cells

1. Harvest and centrifuge cells at low speed. Remove supernatant. (Optional) Wash cells once with cold (4 °C) PBS, centrifuge, and remove the supernatant. Resuspend cells in the residual liquid by flicking the tube.
   ▲ **Note:** After completely removing the supernatant, the cell pellet can be stored at –70 °C.
2. Add Lysis/Binding Buffer.
   Small Scale: 1 mL
   Large Scale: 1 mL per 10^7 cells
   Lyse cells completely by vigorous vortexing for 3–5 minutes.
   ▲ **Note:** A complete lysis is extremely important for further steps.
3. Continue with "Lysate clearance" in section 2.1.1.4.

2.1.1.2 Direct lysis of adherent cells

1. Remove cell culture medium and rinse cells with cold (4 °C) PBS, remove supernatant.
2. Add Lysis/Binding Buffer.
   Small Scale: 1 mL
   Large Scale: 1 mL per 10^7 cells
3. Collect the lysate with a rubber spatula and transfer it into a microfuge tube.
   Lyse cells completely by vigorous vortexing for 3–5 minutes.
   ▲ **Note:** A complete lysis is extremely important for further steps.
4. Continue with "Lysate clearance" in section 2.1.1.4.

2.1.1.3 Detachment and subsequent lysis of adherent cells

1. Remove cell culture medium, rinse cells with PBS, and treat them with trypsin or EDTA solution. When the cells have detached, add culture medium and transfer cells to a centrifuge tube.
2. Centrifuge at low speed and remove supernatant.
   (Optional) Wash cells with cold (4 °C) PBS, centrifuge and remove supernatant. Resuspend cells in the residual liquid by flicking the tube.
   ▲ **Note:** After completely removing the supernatant, the cell pellet can be stored at –70 °C.
3. Add Lysis/Binding Buffer.
   Small Scale: 1 mL
   Large Scale: 1 mL per 10^7 cells
   Lyse cells completely by vigorous vortexing for 3–5 minutes
   ▲ **Note:** A complete lysis is extremely important for further steps.
4. Continue with "Lysate clearance" in section 2.1.1.4.

2.1.1.4 Lysate clearance

1. For more than 5×10^6 cells, if fuzzy material and clumps remain in the lysate, or if the lysate is highly viscous (depending on cell type), **mechanical shearing of DNA** must be performed.
   Transfer lysate to a fresh tube by forcing it 2–5 times with maximum pressure through a 21G needle attached to a 1–5 mL syringe matching the lysate volume. Check that no fuzzy material or clumps remain.

2. (Optional) The foam which is caused during the lysis can be reduced by centrifuging the lysate for 1–3 minutes.
   Small Scale at 13,000×g
   Large Scale at 5,000×g
3. Apply lysate on top of the LysateClear Column that is placed in the centrifugation tube. Centrifuge LysateClear Column.
   Small Scale at ≥13,000×g for 3 minutes
   Large Scale at ≥5,000×g for 10 minutes
   The cleared lysate is collected in the centrifugation tube. LysateClear Columns remove cell debris while the cleared lysate is collected in the centrifugation tube.
4. During centrifugation proceed with column rinse and continue with magnetic labeling and isolation (section 2.2).

2.1.2 Lysis of human, animal, or plant tissue

RNA from tissue tends to degrade quickly, especially when frozen samples thaw. Work fast until tissue is lysed completely. For storage of tissue samples it is recommended to quick-freeze samples in liquid nitrogen and stabilize frozen samples (minimum overnight at –20 °C) in RNA stabilizing buffer. For more information, please contact Technical Support.

Small scale **preparation in µ Columns**
Use a maximum of 30 mg human or animal tissue (spleen: 10 mg, heart: 15 mg, thymus: 5 mg). For many tissues (except, e.g., lung, fat, or skin) 30 mg corresponds to a 3 mm × 3 mm × 3 mm piece. Use up to 100 mg plant tissue.

Large scale preparation in M Columns
Use a maximum of 150 mg human or animal tissue (spleen: 50 mg, heart: 100 mg, thymus: 25 mg). For many tissues (except, e.g., lung, fat, or skin) 150 mg corresponds to a 6 mm × 6 mm × 6 mm piece. Use up to 500 mg plant tissue.

Before starting

▲ Warm Elution Buffer to 70 °C in a heating block. Adjust Lysis/Binding Buffer and Wash Buffer to room temperature.

▲ To achieve a high yield of mRNA, place the heating block close to the Separator and work fast to avoid cooling of the Elution Buffer before applying it to the column.

1. For hard tissue and tissue rich in connective tissue like muscle, heart, bone, and dermis: Grind tissue in a mortar on liquid nitrogen to a homogeneous powder. Prevent thawing of the powder.

   For soft tissue: This can be lysed without grinding.

2. Add Lysis/Binding Buffer.

   Small Scale: 1 ml
   Large Scale: 5 mL

3. Immediately homogenize tissue using an appropriate method such as rotor-stator homogenizer.

   ▲ **Note:** Up to 30 mg tissue can be handled in a 2 mL tube using a small rotor-stator (5 mm diameter).

   ▲ **Note:** A complete lysis is extremely important for further steps.

4. (Optional) The foam which is caused during the lysis can be reduced by centrifuging the lysate for 1–3 minutes.

   Small Scale at 13,000×g
   Large Scale at 5,000×g

5. Apply lysate on top of the LysateClear Column that is placed in the centrifugation tube. Centrifuge LysateClear Column.

   Small Scale at ≥13,000×g for 3 minutes
   Large Scale at ≥5,000×g for 10 minutes

   The cleared lysate is collected in the centrifugation tube.

6. During centrifugation proceed with column rinse and continue with magnetic labeling and isolation (section 2.2).

2.1.3 Sample preparation from whole blood

Small Scale preparation in µ Columns: Use up to 0.5 mL whole blood.
Large Scale preparation in M Columns: Use up to 2.5 mL whole blood.

Before starting

▲ Warm Elution Buffer to 70 °C in a heating block. Adjust Lysis/Binding Buffer and Wash Buffer to room temperature.

▲ To achieve a high yield of mRNA, place the heating block close to the Separator and work fast to avoid cooling of the Elution Buffer before applying it to the column.

1. Transfer peripheral blood (freshly drawn and anti-coagulated) to a suitable tube.

2. Dilute blood with Lysis/Binding Buffer.
   Small Scale: to a final volume of 1 mL
   Large Scale: to a final volume of 5 mL

3. Lyse cells completely by vigorous vortexing for 3–5 minutes.

   ▲ **Note:** A complete lysis is extremely important for further steps.

4. To reduce viscosity of the lysate, **mechanical shearing of DNA** must be performed. Transfer the lysate to a new tube by forcing it 2–3 times with maximum pressure through a 21G needle attached to a 1–5 mL syringe matching the lysate volume. Make sure that no fuzzy material or clumps remain in the lysate.

5. (Optional) The foam which is generated during lysis can be reduced by centrifuging the lysate for 1–3 minutes.
   Small Scale at 13,000×g
   Large Scale at 5,000×g

6. Apply sheared lysate on top of the LysateClear Column that is placed in the centrifugation tube. Centrifuge LysateClear Column.
   Small Scale at ≥13,000×g for 3 minutes
   Large Scale at ≥5,000×g for 10 minutes

   LysateClear Columns remove cell debris while the cleared lysate is collected in the centrifugation tube.

7. During centrifugation, proceed with column rinse and then continue with magnetic labeling and isolation (section 2.2).

2.1.4 For isolation of mRNA from total RNA

Small Scale preparation in µ Columns:
Use a maximum of 200 µg total RNA (max. volume 500 µL).

Large Scale preparation in M Columns:
Use a maximum of 1 mg total RNA (max. volume 2.5 mL).

▲ For best mRNA preparations, use freshly isolated, intact total RNA.

Before starting

▲ Warm Elution Buffer to 70 °C using a heating block. Adust Lysis/Binding Buffer and Wash Buffer to room temperature.

▲ To achieve a high yield of mRNA, place the heating block close to the Separator and work fast to avoid cooling of the Elution Buffer before applying it to the column.

1. Heat total RNA for 5 minutes to 70 °C. Then, chill briefly on ice.

2. Take the tube out of the ice and dilute total RNA with one volume of Lysis/Binding Buffer. If necessary, add Lysis/Binding Buffer to final **minimal volume** of 250 µL for small scale isolation or 1.25 mL for large scale isolation. For examples refer to the table below.

Smale Scale*					
Volume of total RNA [µL]	20	100	150	250	500
Lysis/Binding Buffer [µL]	230	150	150	250	500
Large Scale*					
Volume of total RNA [µL]	200	500	750	1,000	2,500
Lysis/Binding Buffer [µL]	1,050	750	750	1,000	2,500

* Maximal volume of column reservoir: 1 mL for small scale isolation and 3 mL for large scale isolation.

3. Proceed with magnetic labeling and separation (section 2.2).

2.2 Magnetic labeling and isolation

Protocol for the isolation of mRNA

1. Place a MACS Column in the magnetic field of an appropriate MACS Separator.

   µ Column: µMACS Separator or thermoMACS Separator.

   M Column: MiniMACS, OctoMACS, or SuperMACS II Separator.

2. Prepare column by rinsing with Lysis/Binding Buffer and let buffer run through. Columns are "flow stop" and do not run dry.

   µ Column: 100 µL
   M Column: 250 µL

3. Add Oligo(dT) MicroBeads.

   ● For cells, tissues and whole blood:
   50 µL per 1 mL lysate as prepared in section 2.1.

   ● For total RNA:
   25 µL Oligo(dT) MicroBeads per diluted 100 µg total RNA as prepared in section 2.1.4. For less total RNA, also use 25 µL Oligo(dT) MicroBeads.

   Mix by pipetting up and down 2–3 times or vortex shortly. For the hybridization of mRNA to Oligo(dT) MicroBeads, further incubation is not necessary.

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4. Apply lysate on top of the column matrix. Let the lysate pass through. Magnetically labeled mRNA is retained in the column.

5. Rinse column with Lysis/Binding Buffer to remove proteins and DNA. Only for total RNA samples is one single rinse sufficient.

   µ Column: 2×200 µL
   M Column: 3×250 µL

6. Rinse column with Wash Buffer to remove rRNA and DNA.

   µ Column: 4×100 µL
   M Column: 4×250 µL

7. Pre-elution: Apply pre-heated (70 °C) Elution Buffer with a fresh pipet tip for each pipetting step. Discard flow-through.

   µ Column: 27 µL
   M Column: 70 µL

   ▲ **Note:** Discard pipet tip after each dispense. Re-use of one pipet tip for multiple pipetting steps with hot buffer can change the pre-elution volume and thereby reduce the amount of eluted mRNA.

   ▲ **Note:** For a consistent elution volume, *remove* any residual drop at the column tip by touching the column tip with the rim of the RNase-free tube or with an RNase-free pipette tip.

8. Elution: Place a fresh RNase-free tube under the column. Keep the columns in the magnet. Apply pre-heated Elution Buffer.

   µ Column: 50 µL
   M Column: 75 µL

   ▲ **Note:** Collect residual drop at the column tip by touching the column tip with the rim of the RNase-free tube containing the eluate.

   Alternative elution: To increase the mRNA yield by approximately 10%, apply a larger volume of pre-heated Elution Buffer.

   µ Column: 75 µL
   M Column: 100 µL

   ▲ **Note:** The alternative elution will increase the volume of the eluate and decrease the mRNA concentration. Collect residual drop at the column tip by touching the column tip with the rim of the RNase-free tube containing the eluate.

3. Tips & hints

▲ Sample lysis

Incomplete lysis and high viscosity will compromise mRNA yield, slow down column flow, or may cause clogging of the column. If fuzzy material or clumps remain in the lysate, or if the lysate is very viscous, shear sample mechanically: Pass the lysate several times through a 21G needle attached to a 1–5 mL syringe until all clumps are dissolved and viscosity is reduced.

▲ mRNA yield, mRNA purity, and RNA integrity

Please read section 5.2 "Quantification and quality control of RNA".

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4. Troubleshooting

Some lysate is left in the lysate

Clear Column after centrifugation. Centrifuge again. Please do not use more sample material than specified.

Slow gravity flow of columns

The gravity flow of the columns depends on amount, tissue type (e.g. thymus and spleen can be problematic), and viscosity of sample material. Do not overload columns by using unspecified sample amounts which might lead to slowing of gravity flow. Inserting a DNA shearing step will improve gravity flow in the column.

Wash or Elution Buffer does not run into the column

Take off buffer. Use fresh buffer and pipet with force directly on top of column matrix. Alternatively, pipet buffer up and down, avoiding air bubbles.

Low mRNA yield

Scarce mRNA source
The amount of poly(A) RNA (1–5% of total cellular RNA) depends on sample type and physiological state. Expected yields may vary widely.

Incomplete sample lysis and very viscous lysates
Incomplete lysis and highly viscous lysates will compromise mRNA yield, slow down column flow, or may cause clogging of the column. If fuzzy material or clumps remain in the lysate, or if the lysate is very viscous, mechanically shear the sample: Pass the lysate several times

through a 21G needle attached to a 1–5 mL syringe until all clumps are dissolved and viscosity is reduced.

Degraded RNA

Its molecular characteristics make RNA chemically instable and inherently susceptible to ubiquitous RNases. It is therefore recommended to work rapidly without interruptions to minimize mRNA degradation.

Avoid degradation

Sample collection and storage
After sample collection, work quickly until samples are completely lysed, flash frozen in liquid nitrogen or stabilized in RNA stabilization buffer. Do not let frozen tissue thaw unless it is stabilized.

Sample preparation
Always wear disposable gloves. Do not touch the column tip. Place the column in the magnet from the front to avoid contact of the column tip with the magnet. Change pipette tips in case of contact with column housing and between pipetting of different buffers or reagents. Disposable filter tips are recommended.

Sample analysis
Use a positive control (e.g. RNA molecular weight marker or an RNA sample known to be intact) to rule out gel problems.

Low purity of RNA
Increase number of wash steps in case of ribosomal RNA contamination

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Variation in mRNA yield and elution volume

For a consistent elution volume, remove any residual drop at the column tip after pre-elution. After elution, collect any residual drop by touching the column tip with the rim of the tube containing the eluate.

5. Appendix

5.1 In-column removal of DNA traces using DNase I

Traces of genomic DNA in RNA preparations, particularly in total RNA, can interfere with downstream applications such as subtractive hybridization, microarray analysis, real-time PCR, or RACE-PCR. Even when purifying messenger RNA, residual contamination of genomic DNA cannot be totally eliminated. The µMACS mRNA Isolation Technology was developed to obtain highly pure mRNA devoid of genomic DNA and other contaminations. However, even with this procedure minimal amounts of residual genomic DNA cannot be entirely excluded.

To completely eliminate genomic DNA contamination, a short DNase I treatment directly in the column following the µMACS mRNA isolation can be performed. No inactivation or precipitation step is required, as the DNase I is simply washed away from the column.

Reagent and instrument requirements

- RNase-free DNase I (Ambion®, # 2222, 2 U/µL).
  µ Column: 1 µL DNase I / reaction
  M Column: 3 µL DNase I / reaction
- DNase buffer, RNase-free, supplied with DNase I; or prepare standard DNase I buffer, e.g.
  10× DNase I Buffer: 100 mM Tris HCl (pH 7.5)
  25 mM $MgCl_2$
  5 mM $CaCl_2$

Protocol for in-column DNase I treatment

Necessary volumes of 1× DNase I Buffer/reaction.

µ Column: 150 µL
M Column: 400 µL

Perform the magnetic separation as described in section 2.2. After rinsing column with Wash Buffer (step 6) **do not elute** mRNA with pre-heated elution buffer as indicated. Proceed with the following protocol.

1. Apply 1× DNase I Buffer onto the column.
   µ Column: 100 µL
   M Column: 250 µL
2. Prepare DNase I reaction solution.
   µ Column: 1 µL DNase I in 50 µL 1× DNase I Buffer
   M Column: 3 µL DNase I in 150 µL 1× DNase I Buffer
3. Apply DNase I reaction solution onto the column.

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4. Incubate DNase I on column for 1–2 minutes at room temperature.
   ▲ **Note:** Incubation time should not be extended as residual RNase activity in the DNase solution cannot be excluded.
5. Wash column with Lysis/Binding Buffer.
   µ Column: 2×200 µL
   M Column: 3×250 µL
6. Wash column with Wash Buffer.
   µ Column: 4×100 µL
   M Column: 4×250 µL
7. Elute mRNA as described in section 2.2, step 7 and 8.

5.2 Quantification and quality control of RNA

Quantification by measuring UV Absorbance

mRNA yield can be determined by measuring the absorbance (A) at 260 nm, if RNA concentrations >5 ng/µL are expected. The measured A_{260} should have a value of ≥0.1 to ensure reliable analysis. For accurate results with conventional spectrophotometers we recommend the usage of RNase-free disposable cuvettes with a small volume (50 µL), which allow measurement of the undiluted mRNA eluate. An absorbance of 0.1 corresponds to 4 µg RNA/mL (path length: 1 cm). Therefore, the yield of mRNA can be calculated as follows:

$$A_{260} \times 40 \times \text{dilution factor} = \mu g \text{ mRNA/mL}$$

For UV measurements of very small samples, like aliquots of 1 µL volume, instruments of NanoDrop Technologies, e.g. NanoDrop ND-100, can be used.

To reduce the volume of the eluate and to concentrate the mRNA, please refer to section 5.3.

Quantification using fluroscent dye

If RNA concentrations <5 ng/µL are expected, measure dilutions of the mRNA eluates with the Quant-iT™ RiboGreen® RNA quantitation assay (high range from 20 to 1000 pg/µL; low range from 1 to 50 pg/µL, Molecular Probes®), in a fluorescence microplate reader (excitation at 500 nm, emmision at 525 nm). Please follow the instructions of the manufacturer.

Capillary electrophoresis

Using capillary electrophoresis very low concentrations of RNA can be detected. Detection limits of the products range between 200–5,000 pg/µL RNA (Experion™ RNA HighSens Analysis Kit, Bio-Rad Laboratories; RNA 6000 Pico LabChip® Kit, Agilent Technologies) and 25–500 ng/µL RNA (Experion™ RNA StdSens Analysis Kit, Bio-Rad Laboratories; RNA 6000 Nano LabChip® Kit, Agilent Technologies).

mRNA purity

By measuring the absorbance at 280 nm possible protein contamination of the obtained eluate can be determined. The ratio A_{260}/A_{280} should be between 1.8 and 2.2 for pure mRNA.

RNA integrity

To analyze RNA integrity, formaldehyde denaturing agarose gel electrophoresis can be performed, although its sensitivity is very limited. Instead, capillary electrophoresis is the method of choice to evaluate RNA integrity from 1 µL sample (see above). High quality mRNA yields a broad peak with a maximum between the two ribosomal RNA types which are present at low levels. Depending on the tissue type additional peaks can be seen.

5.3 How to concentrate eluted mRNA

A standard procedure to reduce volume of mRNA samples is the usage of a SpeedVac™ instrument. Since RNA is eluted with RNase-free H_2O, there is no danger of concentrating salt or EDTA in the sample.

Another approach to concentrate mRNA is preciptiation, as described in the protocol below.

1. Add 0.1 volume of RNase-free 5 M ammonium acetate or 3 M sodium acetate pH 5.2 and mix.
2. (Optional) Add 1 µL glycogen (20 mg/mL).
   ▲ **Note:** If low amounts of mRNA are precipitated, like <1 µg mRNA, use carriers, such as 20 µg of glycogen or 10 µg of *E. coli* tRNA, to precipitate the mRNA.
3. Add 2.5 volumes of absolute ethanol and mix thoroughly by vortexing.
4. Incubate for 30 minutes at –70 °C or overnight at –20 °C.
5. Centrifuge at ≥13,000×g for 20–30 minutes at 4 °C.
6. Carefully remove and discard the supernatant (attention: the RNA pellet may not adhere tightly to tube).
7. To remove residual salt, add 1 mL RNase-free 75% ethanol and vortex.

8. Centrifuge at ≥13,000×g for 10 minutes at 4 °C.
9. Carefully remove the supernatant and dry the mRNA pellet.
10. Dissolve mRNA in an appropriate volume of buffer or RNase-free water.

6. References

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