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This MACS® product is for *in vitro* research use only and not for diagnostic or therapeutic procedures.

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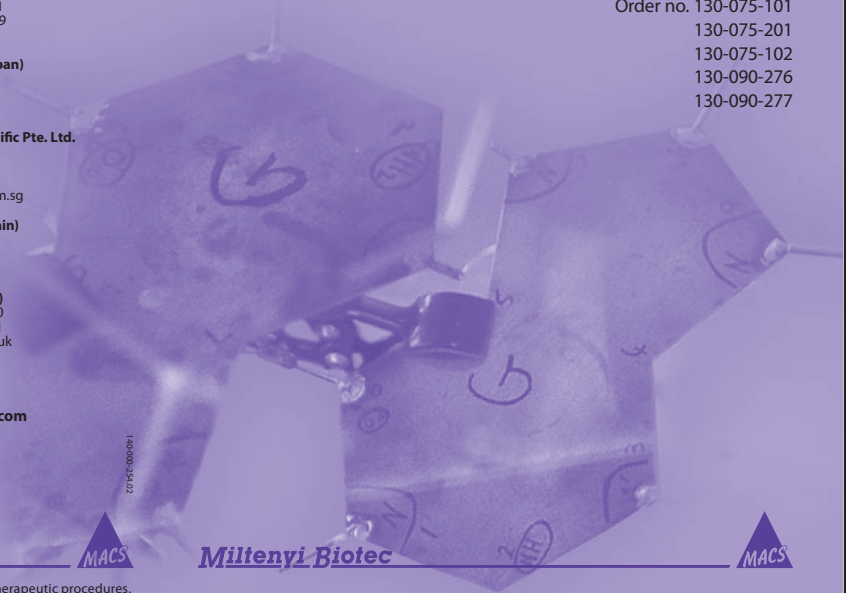
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μMACS™ mRNA Isolation Kits

Order no. 130-075-101
130-075-201
130-075-102
130-090-276
130-090-277



Index

1. Description	3
1.1 Components and size	3
1.2 mRNA isolation with MACS® Technology	4
1.3 Kit capacities	8
1.4 Reagent and instrument requirements	9
1.5 Related products	10
2. Protocols for mRNA isolation	11
2.1 Sample preparation	11
2.2 Magnetic labeling and isolation	20
3. Tips & hints	22
4. Troubleshooting	23
5. Appendix	25
5.1 In-column removal of DNA traces using DNase I	25
5.2 Quantification and quality control of RNA	27
5.3 How to concentrate eluted mRNA	30
6. References	31

The cover photo shows a replica of the DNA model built in 1953 by James D. Watson and Francis Crick at the Cavendish Laboratory in Cambridge.

This model is located at Heureka, the Finnish Science Centre. Photography by Alexander Budde; © Miltenyi Biotec GmbH, Germany. Detailed information on the history of the Watson-Crick model can be found in: de Chadarevian, S. (2003) Relics, replicas and commemorations. Endeavour 27: 75–79.

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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	mRNA Isolation – Small Scale	mRNA Isolation – Small Scale	mRNA Isolation – Large Scale	mRNA Isolation – Large Scale	mRNA Isolation – Total RNA
Order no.	130-075-201	130-090-276	130-075-101	130-090-277	130-075-102
Oligo(dT) MicroBeads	1 mL	0.5 mL	2×1 mL	1 mL	2×1 mL
Lysis/Binding Buffer	40 mL	40 mL	60 mL	40 mL	40 mL
Wash Buffer	20 mL	20 mL	20 mL	20 mL	20 mL
Elution Buffer	2×1.3 mL	1.3 mL	2×1.3 mL	1.3 mL	2×1.3 mL
No. of columns	20 μ Columns	10 μ Columns	8 M Columns	4 M Columns	8 M Columns
LysateClear Columns	20	10	8	4	–
Reactions	20	10	8	4	8

Product format Oligo(dT) MicroBeads: non-sedimenting MicroBeads conjugated to oligo (dT)₂₅. Suspension contains 0.1% SDS.

Lysis/Binding Buffer: a high salt buffer containing 1% SDS.

Wash Buffer: a low salt buffer.

Elution Buffer: RNase-free H₂O.

Small Scale LysateClear Columns (maximal reservoir volume: 1 mL; capacity: lysate from a maximum of 1×10^7 cells, 30 mg animal tissue or 100 mg plant tissue) and 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 μ g mRNA).

M Columns (capacity: up to 50 μ g mRNA).

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

Storage

Store Buffer Set Box containing buffers and MicroBeads protected from light at 4–8 °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 at room temperature, dry and 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 μ MACS™ 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 μ MACS mRNA Isolation Kits full length, intact mRNA can be obtained from fresh, frozen or cultured cells¹, animal^{2,3} or plant tissue, whole blood or total RNA⁴. 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 (see 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, μ MACS™ 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 μ MACSOne-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.

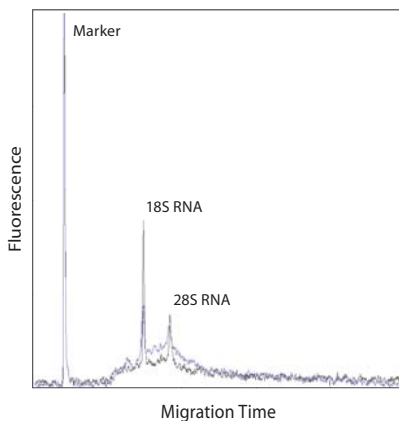


Figure 1: Elution diagram of mRNA

mRNA was isolated either with μ MACS Technology (purple) or latex resin (black) from 200 μ g 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 μ MACS mRNA Isolation Kits

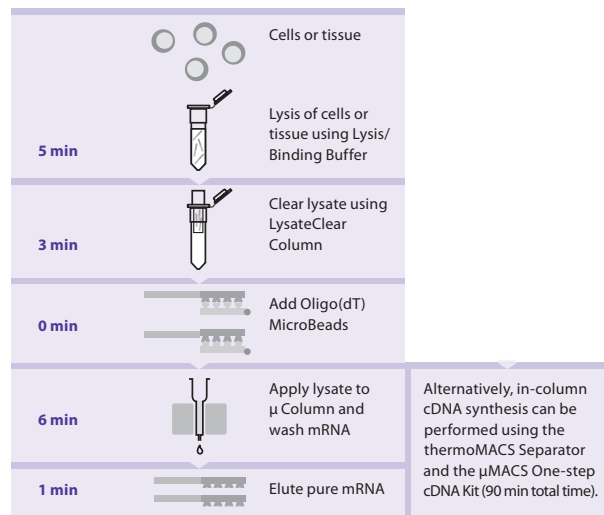


Figure 2: Isolation of mRNA with Oligo(dT) MicroBeads

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 0.5–1 μg. 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.

μMACS mRNA Isolation Kit from total RNA (M Columns)

- Isolation of up to 50 μg mRNA from a maximum of 1 mg total RNA.

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8

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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, VarioMACS™ Separator or SuperMACS™ 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 $\geq 13,000 \times g$ or $\geq 5,000 \times g$, respectively.
- (Optional) Antifoam A reagent (1%), Sigma-Aldrich, can be added to prevent extensive foam formation during sample homogenization.
- (Optional) RNA stabilizing buffer.



9

1.5 Related products

- μMACS One-step cDNA Kit (# 130-091-902)
- μMACS One-step cDNA Starting Kit (# 130-091-989)
- MACS® products for cell separation:
www.miltenyibiotec.com
- PIQOR™ Microarray products and services:
www.miltenyibiotec.com

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 see „Tips & hints“, chapter 3).

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 μMACS Separator and work fast to avoid cooling of the Elution Buffer before applying it to the column.

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10

140-000-254.02

140-000-254.02



11

2.1.1.1 Lysis of suspension cells

1. Harvest and centrifuge cells at low speed. Remove supernatant and (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 PBS 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 $13,000 \times g$

Large scale at $5,000 \times g$

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

Small scale at $\geq 13,000 \times g$ for 3 minutes

Large scale at $\geq 5,000 \times g$ 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 (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 \times 3 mm \times 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 (but not e.g. lung, fat or skin) 150 mg corresponds to a 6 mm \times 6 mm \times 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 μ MACS 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.

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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).

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16

140-000-254.02

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 μ MACS 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



17

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 μ g total RNA (max. volume 500 μ L).

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. Adjust Lysis/Binding Buffer and Wash Buffer to room temperature.
- ▲ To achieve a high yield of mRNA, place the heating block close to the μ MACS 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 μ L for small scale isolation or 1.25 mL for large scale isolation. For examples see table below.

Small scale*	Volume of total RNA (μ L)	20	100	150	250	500
	Lysis/Binding Buffer (μ L)	230	150	150	250	500
Large scale*	Volume of total RNA (μ L)	200	500	750	1,000	2,500
	Lysis/Binding Buffer (μ L)	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).

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18

140-000-254.02

140-000-254.02



19

2.2 Magnetic labeling and isolation

Protocol for the isolation of mRNA

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

μ Column: μMACS Separator or thermoMACS™ Separator.

M Column: MiniMACS™, OctoMACS™, VarioMACS™, or SuperMACS™ Separator.

- 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

- 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.

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

- 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

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

μ Column: 4×100 μL

M Column: 4×250 μL

- 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.

- 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.

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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“.

4. Troubleshooting

Some lysate is left in the LysateClear 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.

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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

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.

Miltenyi Biotec**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 μ MACS 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 μ MACS 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/ μ L).
 μ Column: 1 μ L DNase I / reaction
M Column: 3 μ L DNase I / reaction
- DNase buffer, RNase-free, supplied with DNase I; or prepare standard DNase I buffer, e.g.
 10 \times DNase I Buffer: 100 mM Tris HCl (pH 7.5)
 25 mM MgCl₂
 5 mM CaCl₂

**Protocol for in-column DNase I treatment**

Necessary volumes of 1 \times DNase I Buffer/reaction.

μ Column: 150 μ L

M Column: 400 μ L

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 \times DNase I Buffer onto the column.
 μ Column: 100 μ L
M Column: 250 μ L
2. Prepare DNase I reaction solution.
 μ Column: 1 μ L DNase I in 50 μ L 1 \times DNase I Buffer
M Column: 3 μ L DNase I in 150 μ L 1 \times DNase I Buffer
3. Apply DNase I reaction solution onto the column.
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 \times 200 μ L
M Column: 3 \times 250 μ L
6. Wash column with Wash Buffer.
 μ Column: 4 \times 100 μ L
M Column: 4 \times 250 μ L
7. Elute mRNA as described in section 2.2, step 7 and 8.

Miltenyi Biotec**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/ μ L are expected. The measured A₂₆₀ 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 μ L), which allow measurement of the undiluted mRNA eluate. An absorbance of 0.1 corresponds to 4 μ g RNA/mL (path length: 1 cm). Therefore, the yield of mRNA can be calculated as follows:

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

For UV measurements of very small samples, like aliquots of 1 μ L 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 read section 5.3.

Quantification using fluorescent dye

If RNA concentrations <5 ng/ μ L are expected, measure dilutions of the mRNA eluates with the RiboGreen® RNA quantitation assay (high range from 20 to 1000 pg/ μ L; low range from 1 to 50 pg/ μ L, Molecular Probes), in a fluorescence microplate reader (excitation at 500 nm, emission 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/ μ L RNA (Experion RNA HighSens Analysis Kit, BioRad Laboratories; RNA 6000 Pico LabChip® Kit, Agilent Technologies) and 25–500 ng/ μ L RNA (Experion RNA StdSens Analysis Kit, BioRad 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 μ L 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 precipitation, 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 μ L glycogen (20mg/mL).
▲ **Note:** If low amounts of mRNA are precipitated, like < 1 μ g mRNA, use carriers, such as 20 μ g of glycogen or 10 μ g 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 $\geq 13,000\times g$ 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 $\geq 13,000\times g$ 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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