Miltenyi Biotec

MultiMACS™ mRNA Isolation Kits User manual

MultiMACS mRNA Isolation Kit (12×8) Order no. 130-092-520
MultiMACS mRNA Isolation Kit (4×96) Order no. 130-092-519

Miltenyi Biotec

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Contents

1. Description

1.1 Components and size

Order no.	130-092-520 (12×8)	130-092-519 (4×96)
Oligo(dT) MicroBeads	3×1 mL	4×3×1 mL
Lysis/Binding Buffer	210 mL	4×210 mL
Wash Buffer	100 mL	4×100 mL
Elution Buffer	50 mL	4×50 mL
Multi-8 Columns	12	–
MultiColumn Frame	1	4 (with Multi-96 Columns)
Multi-96 Columns	–	4 (inserted in Deep Well Block)
Deep Well Block, 2.5 mL	1 (with adhesive sealing foil)	4 (with Multi-96 Columns)
Microtiter Plate	1 (with adhesive sealing foil)	4 (with adhesive sealing foil)
Reactions	96	384

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Multi-8 Column Box

12 Multi-8 Columns, separately sterile packaged
1 MultiColumn Frame
1 Deep Well Block, 96×2.5 mL, with adhesive sealing foil
1 Microtiter Plate, U-bottom, with adhesive sealing foil

Multi-96 Column Box

4 Multi-96 Columns, 96×2.5 mL, packaged sterile, inserted into a Deep Well Block
4 Microtiter Plates, U-bottom, with adhesive sealing foil

Multi-8 and Multi-96 Columns cannot be used for cell separations.

Product format

Oligo(dT) MicroBeads: non-sedimenting MicroBeads (diameter 50 nm) conjugated to oligo(dT)$_{25}$; supension contains 0.1% SDS
Lysis/Binding Buffer: a high salt buffer containing 1% SDS
Wash Buffer: a low salt buffer containing NaCl, Tris-HCl, and EDTA
Elution Buffer: RNase-free H_2O

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

Storage

Store reagent box containing buffers and MicroBeads protected from light at 4–8 °C. Do not freeze. The expiration dates are indicated on the vial labels. Store Columns, Deep Well Blocks, and Microtiter Plates 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 up to five percent of the total RNA, many downstream applications such as RT-PCR, Northern blotting, or microarray analysis are performed to analyze mRNA expression.

Hence, accurate gene expression analyses depend on mRNA isolation methods that circumvent common pitfalls: DNA contamination and degradation of the RNA during the isolation procedure 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 MultiMACS™ mRNA Isolation is a robust and reproducible mRNA isolation procedure based on MACS® Technology. The core components are the MultiMACS Separation Unit (Separator), the MACS Columns, and the superparamagnetic Oligo(dT) MicroBeads, which hybridize to the stretch of adenosine residues at the end of eukaryotic mRNA. When a cell lysate with Oligo(dT) MicroBeads is loaded onto a MACS Column placed in a MACS Separator, the magnetically labeled mRNA is retained in the strong magnetic field and can be isolated.

With the MultiMACS mRNA Isolation Kit, 96 mRNA preparations can be performed in 45 minutes directly from cells or tissues. The extremely small MicroBeads, 50 nm in diameter, allow fast reaction kinetics while the column technology provides effective washing steps to minimize DNA or rRNA contamination.

In combination with the use of gravity flow columns, the MultiMACS™

mRNA isolation procedure circumvents disadvantages of other total RNA or mRNA isolation techniques such as unequal samples volumes and leakiness of vacuum manifolds, aerosol formation at centrifugation steps, long incubation times for mRNA hybridization, or time-consuming removal of supernatant as well as bead-carryover with µm-sized magnetic beads.

Using MultiMACS mRNA Isolation Kits, variations of yield and real-time PCR cycle thresholds are generally lower (figure 1 and 2), cross contamination is prevented (figure 3) and genomic DNA contamination is significantly reduced versus standard 96-well total RNA isolation kits. The flexible Multi-8 Column strip format allows from eight to 96 samples to be processed in one experiment. As the Multi-8 or Multi-96 Columns use the standard microtiter plate-format, the MultiMACS Separator can be used either semi-automated on the bench-top or fully automated in a liquid-handling robot.

1.3 Kit capacities

The MultiMACS™ mRNA Isolation Kit is for isolation of mRNA from a maximum of 1×107 cells, 30 mg human and animal tissue (spleen: 10 mg, thymus: 5 mg), or 200 µg total RNA.

For stabilized whole blood samples, see the respective special protocol on the website www.miltenyibiotec.com.

1×107 cells or 30 mg human or animal tissue typically yield 1–6 µg mRNA. Some resting cells may contain significantly lower amounts of mRNA. 96 samples can be processed in less than 45 minutes with an 8-channel pipette.

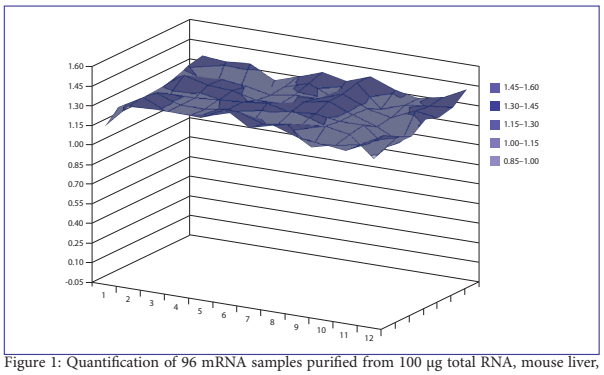


Figure 1: Quantification of 96 mRNA samples purified from 100 µg total RNA, mouse liver, with the MultiMACS™ mRNA Isolation Kit using the MultiMACS Separator. Average yield: 1.3 µg mRNA; coefficient of variation of 96 samples: 5.2%.

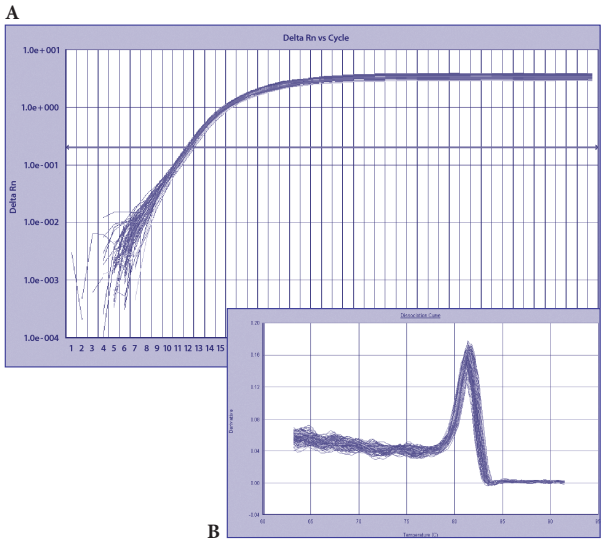


Figure 2: Quantitative RT-PCR of 96 samples purified from 100 µg total RNA, mouse liver, with the MultiMACS™ mRNA Isolation Kit using the MultiMACS Separator. After reverse transcription, quantitative amplification of the housekeeping gene GAPDH was performed (A). Mean cycle threshold: 12.0; coefficient of variation: 2.0%. Display of the dissociation curves of all 96 samples shows pure peaks (B).

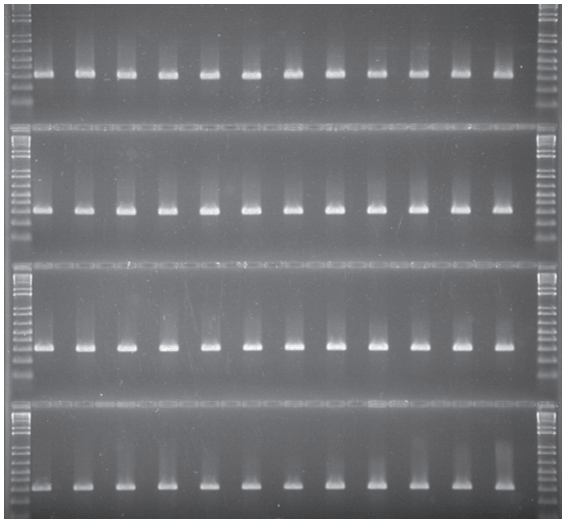


Figure 3: RT-PCR products were generated from 1 µL eluate using GAPDH primers (35 cycles) and then separated by gel electrophoresis in a 2% agarose gel. mRNA was purified with the MultiMACS™ mRNA Isolation Kit using the MultiMACS Separator in a chessboard pattern: 15 mg mouse liver alternated with negative controls containing Lysis/Binding Buffer. The first row shows MultiMACS Separator positions A1–12, B12–1 of the MultiMACS-96 Columns; the second row C1–12, D12–1; the third row E1–12, F12–1; and the fourth row G1–12, H12–1, respectively. Molecular weight markers are in the first and last lane of every row.

1.4 Reagent and instrument requirements

All additionally required equipment must be RNase-free.

- MultiMACS™ 96 Separation Unit
- Heating block plus reagent reservoir compatible with 8-well or 96-well (8/96-well) format for heating of Elution Buffer to 70 °C, e.g. thermocycler, heating block, or thermoshaker compatible with 8/96-well format
- Further microtiter plates or 8-well strips with caps

For homogenization and lysis of up to 10^4 cells in a microtiter plate

- Microplate-shaker

For homogenization and lysis of whole blood, tissue, and more than 10^4 cells

- Multi-96 Filter or Multi-8 Filter plus Frame
- Bead mill, e.g. Mini-Bead Beater-96, BioSpec Products, or TissueLyser, Qiagen
- Stainless steel beads for parallel lysis/homogenization (see supplier of bead mill)

For homogenization of tissue

- Centrifuge compatible with tubes containing lysate, e.g. 2 mL tubes or 8-well strips

For manual use

- 8-channel pipette with tips, e.g. 8-Channel Impact® Pipettor from Matrix Technologies, Corporation:
  - i) Volume range 15–1250 µL with 1250 µL TallTip (102 mm) Filter Tips for transferring lysate and dispensing wash buffers quickly without foaming
  - ii) Volume range 5–250 µL for dispensing hot elution buffer in a single pipetting mode
- Two disposable reagent reservoirs for multichannel pipettes as reservoirs for Lysis/Binding Buffer and Wash Buffer

For automated use

- Liquid handling platform with four to eight pipetting channels, range 30 µL to 1 mL, and a gripper tool to grip plates sideways
- MultiMACS™ Adapter and command line interface mumcli.exe (please contact technical support)
- Reservoir holder and reagent reservoirs for Lysis/Binding Buffer, Wash Buffer, and MicroBeads

1.5 Related products

- MultiMACS™ 96 Separation Unit (# 130-091-937)
- Multi-8 Columns (# 130-092-444)
- Multi-96 Columns (# 130-092-445)
- Multi-8 Filter (# 130-092-546)

- Multi-96 Filter (# 130-092-547)
- Multi-8 Filter and Frame (# 130-092-548)
- Deep Well Block (DWB, 2.5 mL, with adhesive sealing foil, # 130-092-549)
- PrepProtect (100 mL, # 130-092-642; 10 mL, # 130-092-643), for details see Tips & hints
- MACS® products for cell separation: www.miltenyibiotec.com
- PIQOR™ microarray products and services: www.miltenyibiotec.com

2. Protocols for mRNA isolation

The MultiMACS™ Separation Unit provides a list of pre-defined separation programs to choose from (please visit www.miltenyibiotec.com for updates). The standard program for Multi-8 or Multi-96 Columns is called MULTI-8/96 POS.

▲ **Caution: Read the MultiMACS Separator User Manual carefully before running a process. Read the section Warnings and precautions before switching on the instrument.** Always be sure that the MultiMACS 96 Magnet, the MultiMACS Column Holder, and the plates are in the same orientation (see MultiMACS Separator User Manual for details).

▲ Thorough sample homogenization and cell lysis, as well as the reduction of viscosity of lysates, are very important to avoid clogging of the columns.

▲ To run a process with different process parameters, a new program can be created or the parameters of an existing one can be edited. Please see details in the corresponding sections of the MultiMACS Separator User Manual.

▲ All additionally required equipment must be RNase-free.

Before starting

▲ Adjust Lysis/Binding Buffer and Wash Buffer to room temperature. Warm the Elution Buffer to 70 °C in a heating device with a compatible reagent reservoir in 8-well format, e.g. thermocycler, heating block, or thermoshaker compatible with 8/96-well format.

▲ The Pre-elution volume has to be exact. Please calibrate an 8-channel pipette before use as described in section 2.4.

▲ If purifying mRNA from tissues or >10^4 cells, use Multi-8 Filter for up to 48 samples and Multi-96 Filter for more than 48 samples (not included in the kit, see Reagent and instrument requirements).

▲ If using plates with other than standard height dimensions, use plates that comply to the ANSI/SBS standards and adjust the process parameter Plate height, see MultiMACS™ Separator User Manual.

For purification of less than 96 samples

▲ If working with the Multi-8 Filter Frame, be aware that only every second row can be used.

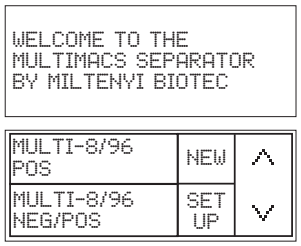
▲ Unpack the necessary amount of individual, sterile-packed Multi-8 Columns and put them in the MultiColumn Frame (use the same pattern as for the Multi-8 Filter). Avoid touching the column tips.

For purification of 96 samples

▲ Unpack Multi-96 Columns. Avoid touching the column tips.

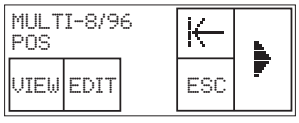
2.1 Start program of the MultiMACS™ 96 Separation Unit

1. Switch on the instrument and touch the Welcome Screen or wait for a few seconds until the Process Selection Screen appears.



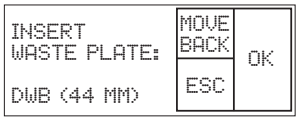
▲ Note: The scroll function is only visible if there are more programs than displayed.

2. The last process performed on the MultiMACS™ is displayed on the upper left segment (default: MULTI-8/96 POS). The one but last process is listed below. If necessary, scroll through the list of available process names by touching the symbol ^ or v until MULTI-8/96 POS is displayed. Touch **MULTI-8/96 POS** to go to the Process Management Screen.

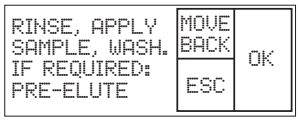


3. If necessary, check the process parameters (see MultiMACS™ Separator User Manual). Touch ▶ to start the process and to move the magnet to the start position. Follow the instructions given on the Touch Display.

4. Insert a MultiColumn Frame with up to twelve Multi-8 Columns or a pre-packed Multi-96 Column into the MultiMACS Column Holder. Touch **OK** to move the magnet upwards. The following screen appears.



5. Place the waste plate, e.g. Deep Well Block (DWB, 2.5 ml), onto the Tip-Touch Plate. If using a plate with a different height, adjust the process parameter Plate height for the waste plate (see corresponding section in the MultiMACS Separator User Manual).
Touch **OK** to move the MultiMACS™ 96 Magnet downwards. Column tips now slightly immerse in the waste plate. The next screen appears.



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6. Rinse columns with 100 µL Lysis/Binding Buffer and let buffer run through. Columns are "flow stop" and do not run dry.

7. Proceed with section 2.2.

2.2 Sample preparation and magnetic labeling

MultiMACS mRNA isolation is compatible with the following sample types: suspension and adherent cell samples (section 2.2.1), human and animal tissue (section 2.2.2). Also, it is used for isolation of mRNA from total RNA (2.2.3). For mRNA isolation from whole blood, please refer to the respective special protocol on the website www.miltenyibiotec.com.

2.2.1 Lysis and magnetic labeling of cells

2.2.1.1 Lysis and magnetic labeling of suspension cells

For up to 10^4 cells

▲ A DNA shearing step is generally not necessary.

1. Transfer up to 10^4 cells to each well of a standard 96-well microplate. Centrifuge for 5 min at 300×g and aspirate the entire supernatant.
2. Premix 30 µL Oligo(dT) MicroBeads and 200 µL Lysis/Binding Buffer per well. Add mixture (230 µL) to each well containing a cell pellet, and shake at 750 rpm for 5 min in a microplate shaker. If no microplate shaker is available, lyse cells by pipetting up and down.
   ▲ **Note:** A complete lysis is extremely important for further steps.
3. Apply lysate on top of the Multi-8/96 Columns matrix. Let the lysate

pass through the Multi-8/96 Columns. Magnetically labeled mRNA is retained in the columns.

4. Proceed with section 2.3.

For up to 5×10^5 cells

▲ A DNA shearing step is generally not necessary. To avoid the risk of clogging the column, the use of a filter is important. During this protocol, a Multi-96 Filter or up to six Multi-8 Filter in a Multi-8 Filter Frame and (optionally) a Deep Well Block (2.5 mL), not supplied with the kit, are used.

▲ If working with the Multi-8 Filter Frame, be aware that only every second row is usable.

1. Unpack and place a filter tool (Multi-96 Filter or up to six Multi-8 Filter in a Multi-8 Filter Frame) on top of the Multi-8/96 Columns.
2. Transfer up to 5×10^5 cells to a tube or to a well of a Deep Well Block. Centrifuge for 5 min at 300×g and aspirate the supernatant completely. Resuspend cell pellet by vortexing it shortly.
3. Premix 30 µL Oligo(dT) MicroBeads and 1000 µL Lysis/Binding Buffer per well. Add mixture (1030 µL) to each well containing a cell pellet, and lyse cells completely by vigorous vortexing. Alternatively, lyse cells by pipetting up and down several times.
   ▲ **Note:** A complete lysis is extremely important for further steps.
4. Apply lysate on top of the Multi-8/96 Filter, placed on the Multi-8/96 Columns. Let lysate pass through.
   ▲ **Note:** Passage of lysate may take 2–10 min.

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5. Apply 200 µL Lysis/Binding Buffer onto Multi-8/96 Filter. Remove drops from the tips of the Multi-8/96 Filter by touching the inner wall of the Multi-8/96 Columns, and discard Multi-8/96 Filter. Magnetically labeled mRNA is retained in the Multi-8/96 Columns.
6. Proceed with section 2.3.

For up to 10^7 cells

▲ To reduce the viscosity of the lysate, mechanical shearing of DNA must be performed, e.g. with a bead mill as described below. To avoid the risk of clogging the column when processing more than to 5×10^6 cells, using a filter is important, such as Multi-96 Filter, or up to six Multi-8 Filter in a Multi-8 Filter Frame.

▲ If working with the Multi-8 Filter Frame, be aware that only every second row can be used.

1. Transfer up to 10^7 cells to an appropriate container, e.g. 8-well strip. Centrifuge for 5 min at 300×g and aspirate supernatant completely.
2. Premix 30 µL Oligo(dT) MicroBeads and 1000 µL Lysis/Binding Buffer per well.
3. Lyse samples with a bead mill, e.g. in an 8-well format: Add one stainless steel bead and mixture (1030 µL) to each cell pellet, close the 8-well strips with caps, and lyse cells immediately by starting the bead mill at maximum frequency for 1–3 min. Remove cap strips carefully.
4. **For up to 5×10^6 cells:** Apply lysate onto the Multi-8/96 Columns matrix. Wait until the lysate has passed through the Multi-8/96 Columns. Magnetically labeled mRNA is retained in the columns.

For 5×10^6–10^7 cells: Unpack and place a Multi-96 Filter or up to six Multi-8 Filter in a Multi-8 Filter Frame on top of the Multi-8/96 Columns to avoid the risk of clogging the column.

Apply lysate on top of the Multi-8/96 Filter placed on the Multi-8/96 Columns. Let the lysate pass through. Magnetically labeled mRNA is retained in the Multi-8/96 Columns. Apply 200 µL Lysis/Binding Buffer onto the Multi-8/96 Filter.

Remove drops from the tips of the Multi-8/96 Filter by touching the inner wall of the Multi-8/96 Columns and discard Multi-8/96 Filter. Magnetically labeled mRNA is retained in the columns.

5. Proceed with section 2.3.

2.2.1.2 Lysis and magnetic labeling of adherent cells

For up to 10^4 cells grown in a microtiter plate

1. Aspirate entire cell culture medium.
2. Premix 30 µL Oligo(dT) MicroBeads and 200 µL Lysis/Binding Buffer per well. Add mixture (230 µL) to each well containing cells and shake at 750 rpm for 5 min in a microplate shaker. If no shaker is available, lyse by pipetting up and down.

   ▲ **Note:** A complete lysis is extremely important for further steps.
3. Apply lysate on top of the Multi-8/96 Columns matrix. Let the lysate pass through. Magnetically labeled mRNA is retained in the columns.
4. Proceed with section 2.3.

Up to 10^7 cells in other culture formats

▲ It is recommended to detach cells, e.g. by trypsinization, for parallel lysis (in an 8-well format).

1. Transfer detached cells to appropriate tubes, e.g. 8-well strips, centrifuge for 5 min at 300×g, and remove the entire supernatant.
2. Wash cell pellet once with cold PBS and proceed with lysis as described for suspension cells, section 2.2.1.1, For up to 10^{7}cells.

2.2.2 Lysis and magnetic labeling of tissue samples

▲ RNA from tissue tends to degrade quickly, especially when frozen samples thaw. Work quickly until tissue is lysed completely. It is highly recommended to stabilize fresh or frozen tissue samples in an appropriate solution, such as PrepProtect (see chapter three, Tips & hints). Samples can be lysed individually with a rotor-stator homogenizer or in parallel with a bead mill described in detail below.

1. When stabilizing with PrepProtect: Remove PrepProtect.
2. Transfer samples into 8-well strips. Put two stainless steel beads (diameter 5 mm) in each well, add 900 µL Lysis/Binding Buffer, and close 8-well strips with cap strips.
3. Lyse samples immediately by starting the bead mill on the highest setting for 5 min. Use a maximum of 30 mg tissue (spleen: 10 mg, thymus: 5 mg). For many tissues (except e.g. lung, fat, or skin), 30 mg corresponds to a piece of approximately 3 mm × 3 mm × 3 mm.

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

4. Centrifuge 8-well strips for 3 min at 6,000×g. Prepare a 96-well plate by pipetting 30 µL Oligo(dT) MicroBeads per sample into the necessary number of wells. Transfer supernatant into these wells and mix by pipetting up and down three times.

   ▲ **Note:** Depending on the amount and type of tissue (not applicable for spleen, thymus, and lung) it might be possible to transfer lysates directly onto the filter tool without prior centrifugation. Overloading will clog only the Multi-8/96 Filter, not the Multi-8/96 Columns.

   ▲ **Note:** Do not centrifuge the lysate-Oligo(dT) Microbead mixture.
5. Apply lysate on top of the Multi-8/96 Filter placed on the Multi-8/96 Columns. Wait 2–10 min to let the lysate pass through the Multi-8/96 Filter.

   ▲ **Note:** A filtration step is not necessary when tissue lysate is perfectly cleared from particles by centrifugation before addition of Oligo(dT) Microbeads. Do not overload columns, especially when working with spleen or thymus.
6. Wash Multi-8/96 Filter once with 200 µL Lysis/Binding Buffer. Remove drops from the tips of the Multi-8/96 Filter by touching the inner wall of the Multi-8/96 Columns and discard Multi-8/96 Filter.
7. Let lysate pass through the columns. Magnetically labeled mRNA is retained in the Multi-8/96 Columns.
8. Proceed with section 2.3.

2.2.3 mRNA from total RNA

Use a maximum of 200 µg total RNA (maximum volume in tubes: 500 µL, in wells of microtiter plates: 80 µL).

▲ A lysis/homogenization step is not necessary.

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

1. Incubate total RNA for 5 min at 70 °C. Then, chill briefly on ice.
2. Take tubes out of the ice and dilute total RNA with at least one volume of Lysis/Binding Buffer (see table below).

Volume of total RNA (µL)	10	20	30	40	50	60	70	80	90 – 500
Lysis/Binding Buffer (µL)	160	150	140	130	120	110	100	90	One volume

▲ **Note:** Maximum volume (total RNA sample and Lysis/Binding Buffer) should not exceed 1 mL.

▲ **Note:** Oligo(dT) MicroBeads can be premixed with Lysis/Binding Buffer, mixture should not be stored or centrifuged.

3. Add 30 µL Oligo(dT) MicroBeads. Mix and apply sample on top of the Multi-8/96 Column matrix. Wait until the liquid has passed through. Magnetically labeled mRNA is retained in the Multi-8 Columns.
4. Proceed with step 2.3.

2.3 Wash

▲ Pipette buffers in a multidispense mode, one aspiration for all dispenses, to avoid foaming.

▲ Always wait until the buffer has completely passed through before applying the next aliquot of buffer.

1. Rinse Multi-8/96 Columns with 2 × 200 µL Lysis/Binding Buffer. Only

for total RNA samples one single rinse is sufficient.

2. Rinse Multi-8/96 Columns with 4×100 µL Wash Buffer.

2.4 Pre-elution

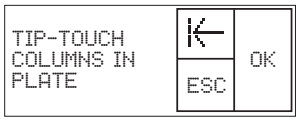
▲ A pre-elution step allows small and reproducible volumes of eluate because it replaces the void volume of the column.

1. Apply exactly 30 µL pre-warmed Elution Buffer (70 °C) onto the Multi-8/96 Column matrix and let buffer pass through completely.

   ▲ Note: Reusing the same pipette tips for multiple pipetting steps with hot buffer will raise the pre-elution volume. Therefore, reduce the volume to approximately 27 µL, pipette the first four dispenses to a waste plate, and then start pipetting onto the column matrix in a single pipetting mode. Alternatively, set volume to 30 µL and discard tips after each dispense.

   ▲ Note: Immerse the tips only slightly (1 mm) into the Multi-8/96 Columns without touching the inner walls of the cavity. Pipette the buffer with high speed onto the centre of the column matrix. No drops should remain at the tip after dispensing.

2. Touch **OK** and the following screen appears.



3. Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the Deep Well Block touch the tips of the Multi-8 Columns. Thereby, any drop on the column tips that did not fall off by gravity will be removed.

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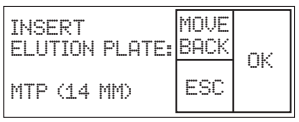
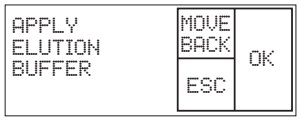
2.5 Elution

1. Touch **OK** to move the MultiMACS™ 96 Magnet upwards. The next screen appears.



2. Remove Deep Well Block. If some wells were unused, aspirate waste liquid and store plate (optionally) covered with sealing foil. Insert the elution plate (Microtiter Plate).

3. Touch **OK** and the MultiMACS 96 Magnet will move downwards until column tips slightly immerse in the elution plate. The following screen appears.



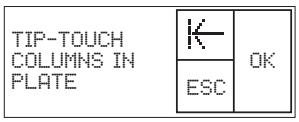
4. Apply 50 µL pre-warmed Elution Buffer (70 °C) directly onto the Multi-8/96 Column matrix. Let the buffer pass through.

   ▲ Note: Reuse of pipette tips for multiple pipetting steps with hot buffer will raise the pre-elution volume; therefore, pipette the first four dispenses to a waste plate and then start pipetting onto the column matrix in a single pipetting mode. Alternatively, discard tips after each dispense.

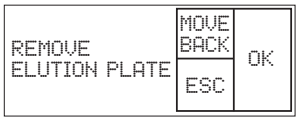
▲ Note: To increase of mRNA yield by 10%, apply 75 µL Elution Buffer. This will lead to decreased mRNA concentration.

▲ Note: Immerse the tips only slightly (1 mm) into the Multi-8/96 Columns without touching the inner walls of the cavity. Pipette the buffer with high speed onto the centre of the column matrix. No drops should remain at the tip after dispensing.

5. Touch **OK** and the next screen appears.



6. Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the elution plate touch the tips of the Multi-8 Columns. Thereby, any drop on the column tips that did not fall off by gravity will be removed. Touch **OK** to move the MultiMACS™ 96 Magnet upwards.



7. Remove Elution Plate. The mRNA can be subjected to downstream applications immediately. Alternatively, seal the plate with adhesive foil (included in the kit) and store it at –20 °C or –80 °C.
   Touch **OK** to move the MultiMACS 96 Magnet away from the Column Holder.

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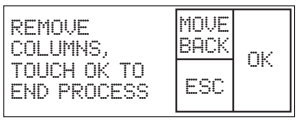

8. Remove MultiColumn Frame with Multi-8/96 Columns. If less than twelve Multi-8 Columns were used, remove the used Multi-8 Columns and store the MultiColumn Frame. Touch **OK** to finish the process.

3. Tips & hints

3.1 Avoid foaming

During homogenization
Add Antifoam A (1%) to Lysis/Binding Buffer. Alternatively, the foam after lysis of samples can be reduced by centrifugation of the lysate.
▲ Note: Do not centrifuge lysate with MicroBeads.

During wash steps
Dispense Lysis/Binding Buffer and Wash Buffer in a multi-dispense mode, one aspiration for all dispenses.

3.2 RNA stabilization in cell or tissue samples

PrepProtect is a non-toxic solution that stabilizes fresh or frozen samples.

Stabilization of fresh samples
The reagent PrepProtect allows samples to be stored or shipped at temperatures above –20 °C. After stabilization of freshly dissected tissues

or cultured cells in PrepProtect, samples can be stored in PrepProtect up to one day at 37 °C, up to one week at 25 °C, up to one month at 4 °C, or for archieval storage below –20 °C.

Maximum stabilization time depends on the RNase content of cells or tissue and might vary. As stabilization of tissue is limited by the penetration of the buffer, cut the tissue into slices with a maximum thickness of 5 mm. For RNA preparation, remove PrepProtect completely, add Lysis/Binding Buffer, and homogenize according to protocol. Incubate for 5 min at 70 °C after lysis. Then, let lysate cool down to room temperature and mix with 30 µL Oligo(dT) MicroBeads.

Stabilization of already quick-frozen tissue
PrepProtect stabilizes frozen samples at –20 °C, thus allowing samples to thaw for a limited time without degradation. Samples become sliceable and can be handled up to one hour at room temperature or overnight at 4 °C without degradation. Therefore, frozen samples after stabilization in PrepProtect can be cut, weighed, and lysed by mechanical tools without prior crushing with mortar/pestle.
For RNA preparation, remove PrepProtect completely, add Lysis/Binding Buffer, and homogenize according to protocol. No additional step is necessary.

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4. Troubleshooting

4.1 Low mRNA yield

Scarce mRNA source
The amount of poly(A) RNA (1–5% of total 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 column. If fuzzy material or clumps remain in the lysate, or if the lysate is very viscous, mechanically shear sample: Most types of tissues can be lysed by using the bead mill with two stainless steel beads (diameter 5 mm) at the highest frequency, e.g. 30 Hz. It is not recommended to lyse very hard tissues such as uterus, breast tumor tissue, and bones with a bead mill. When lysing cells or tissue which were stabilized in PrepProtect prior to freezing, heat these lysates for 5 min at 70 °C and allow to cool to room temperature for another 10 min.

Degraded RNA
Its molecular characteristics make RNA chemically instable and inherently susceptible to ubiquitous RNases. See recommendations to minimize mRNA degradation.

4.2 Avoid degradation

Sample collection and storage
After sample collection, work quickly until samples are completely lysed,

quick-frozen in liquid nitrogen or stabilized in PrepProtect. Do not let frozen tissue thaw unless it is stabilized in PrepProtect. To ensure a good penetration by PrepProtect, the tissue samples must be cut into pieces of maximum 5 mm in diameter.

Sample preparation
Always wear disposable gloves. Do not touch the column tip. Change pipette tips in case of contact with column housing and between pipetting of different buffers or reagents. Disposable filter tips and reagent reservoirs, e.g. Disposable Reagent Reservoirs from Matrix Technologies Corp., are recommended.

Sample analysis
Use a positive control to rule out problems with the analysis procedure, e.g. RNA molecular weight marker or an RNA sample known to be intact.

4.3 Variation in mRNA yield and elution volume

Inconsistent 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 well containing the eluate.

Unequal tissue samples
mRNA contents might vary within different sample types.

Inconsistent sample input
Cell numbers or tissue weights may have not exactly been measured.

Pre-elution/elution step
Do not pour hot Elution Buffer in a non-heated reservoir as the buffer will

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cool down quickly. Pre-elution volume has to be exactly 30 µL. As the dispensed hot buffer volume increases after several dispenses, skip the first four dispenses, check the volume of the fifth dispense, and adjust the volume.

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

4.4 Cross contamination

During bead milling
Use appropriate plastic ware, e.g. 8-well strips with caps, that are not leaky during the homogenization process in the bead mill. After homogenization with the bead mill, centrifuge samples before opening the tubes or strips carefully.

Clogging of column/filter
Clogging of columns has to be avoided. Ensure that the viscosity of the lysate is reduced by a DNA shearing step. In addition, use Multi-8/96 Filter on top of Multi-8/96 Columns to avoid clogging of Multi-8/96 Columns. If using tissue, centrifuge lysates and apply only the supernatant onto the Multi-8/96 Filter. Do not use unspecified sample amounts since this might lead to clogging of columns or filters.

5. Appendix

5. Appendix

5.1 Quantification and quality control of RNA

UV Absorbance

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.

Quantification using fluroscent dye

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

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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, BioRad Laboratories; RNA 6000 Pico LabChip® Kit, Agilent Technologies) and 25–500 ng/µL 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 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.

5.2 Buffer amounts

The kit provides sufficient amount of buffers. Do not store unused buffer in the reservoir or fill it back into the buffer flask. See the table below for the theoretically needed buffer volumes (mL) for the experiment and calculate

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a surplus of approximately 10–20% depending on the void volume of the reagent reservoir.

Number of preps	Micro-Beads (mL)	Lysis/ Binding Buffer[1] (mL)	Lysis/ Binding Buffer[2] (mL)	Lysis/ Binding Buffer[3] (mL)	Wash Buffer (mL)	Elution Buffer[4] (mL)
8	0.24	11.2	12.8	4.8	3.2	8×0.4
16	0.48	22.4	25.6	9.6	6.4	8×0.48
24	0.72	33.6	38.4	14.4	9.6	8×0.56
32	0.96	44.8	51.2	19.2	12.8	8×0.64
40	1.2	56.0	64.0	24.0	16.0	8×0.72
48	1.44	67.2	76.8	28.8	19.2	8×0.8
56	1.68	78.4	89.6	33.6	22.4	8×0.88
64	1.92	89.6	102.4	38.4	25.6	8×0.96
72	2.16	100.8	115.2	43.2	28.8	8×1.04
80	2.4	112.0	128.0	48.0	32.0	8×1.12
88	2.64	123.2	140.8	52.8	35.2	8×1.2
96	2.88	134.4	153.6	57.6	38.4	8×1.28

[1] Sample lysed in 1 mL, no Multi-8/96 Filter.
[2] Sample lysed in 1 mL, Multi-8/96 Filter is used.
[3] Sample is lysed/diluted in 0.2 mL, no Multi-8/96 Filter.
[4] For 50 µL eluate, Elution Buffer has to be applied with an 8-channel pipette from a heatable container. Four dummy dispenses are already included in the volume.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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