Miltenyi Biotec

MultiMACS™ cDNA Synthesis Kits

MultiMACS™ cDNA Synthesis Kit (12×8)	130-094-410
MultiMACS cDNA Synthesis Kit (4×96)	130-094-408

Miltenyi Biotec

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1. Description

1.1 Components and size

MultiMACS cDNA Synthesis Kit Order no.	**(12×8)** 130-094-410	**(4×96)** 130-094-408
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
Equilibration/Wash Buffer	200 mL	200 mL
Resuspension Buffer for Enzyme Mix	10 mL	2×10 mL
cDNA Release Solution	10 mL	2×10 mL
cDNA Elution Buffer	20 mL	2×20 mL
Lyophilized Enzyme Mix*	96 reactions	4×96 reactions
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)
Number of reactions	96	384

* Mix contains reverse transcriptase and dNTPs.

▲ All buffers and MACS Columns included in the MultiMACS cDNA Synthesis Kits are evaluated for the absence of RNase activity.

Product format

Oligo(dT) MicroBeads: non-sedimenting, superparamagnetic MicroBeads (diameter 50 nm) conjugated to oligo(dT)25; supension contains 0.1% SDS

Lyophilized Enzyme Mix: after resolubility ready-to-use enzyme mix

Multi-8 Column Box

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

Multi-96 Column Box
4 Multi-96 Columns, packaged sterile, inserted into a Deep Well Block (96×2.5 mL)
4 Microtiter Plates, U-bottom

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

Storage

Store the two reagent boxes containing buffers, MicroBeads, and lyophilized Enzyme Mix at 2–8 °C. Do not freeze. The expiration dates are indicated on the vial labels.
Store column box containing Multi-8/96 Columns, Deep Well Blocks, and Microtiter Plates at room temperature, dry, and protected from light.

1.2 From cells and tissue to mRNA and cDNA in one step with MACS® Technology

Eukaryotic messenger RNA (mRNA) is the basis to gain information about specific gene expression profiles in cells and tissue. Although mRNA represents only up to five percent of the total RNA, many downstream applications such as real-time PCR, Northern blotting, cDNA synthesis, or microarray analysis are performed to analyze mRNA expression.
Hence, accurate gene expression analyses depend on rapid 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.
In combination with the MultiMACS M96thermo Separation Unit, the MultiMACS cDNA Synthesis Kits allows the 96-well, parallel mRNA isolation, cDNA synthesis, and cDNA purification. All steps are done in the same column; thus, preventing sample contamination and loss of material.[1]

The compact benchtop MultiMACS M96thermo Separator can be operated manually or, when integrated in a robotic pipetting system, fully automated.

Robust and reproducible procedure based on MACS Technology
Here, the core components are the MultiMACS M96thermo Separator, the Multi-8 or Multi-96 Columns, and the superparamagnetic Oligo(dT) MicroBeads, which hybridize to the poly(A) residues at the 3' end of eukaryotic mRNA. The extremely small (50 nm), non-sedimenting MicroBeads possess very fast reaction kinetics and instantly bind to their target molecules. Even from very small samples, mRNA can successfully be isolated.

First, Oligo(dT) MicroBeads are added to the cell lysate, where they bind to the poly(A) residues of mRNA molecules. Then, the labeled lysate is loaded onto a Multi-8 or Multi-96 Column, which is placed in the magnetic field of a MultiMACS M96thermo Separator. The magnetically labeled mRNA is retained within the column while other cell components are removed by stringent washing steps. This technology circumvents disadvantages of other RNA isolation techniques such as leakiness of vacuum manifolds, aerosol formation during centrifugation steps, or time-consuming removal of supernatants or bead carryover with µm-sized magnetic beads.
For direct, in-column cDNA synthesis, a ready-to-use enzyme mix is provided. The mix contains all reagents for an efficient cDNA synthesis, such as dNTPs and highly activated reverse transcriptase, ensuring a maximum yield of full-length cDNA. As the cDNA is synthesized and purified in the same column, no further purification steps are required and loss of target is minimal.

Using MultiMACS cDNA Synthesis Kits, variations of yield are generally reduced, and real-time PCR cycle thresholds are generally lower as crossing points are reached earlier presumably due to the increase in mRNA and cDNA yield (fig. 1). In addition, cross contamination is prevented (fig. 2), and genomic DNA contamination is significantly reduced versus standard 96-well total RNA isolation kits.

A)

B)

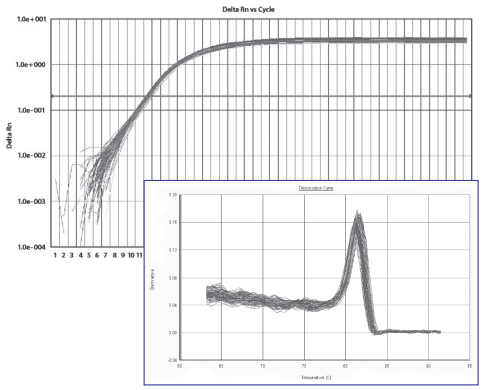


Fig. 1: Quantitative PCR of 96 cDNA samples generated with the MultiMACS System
cDNA was synthesized from 5 µg of total RNA of Molt-4 cells with the MultiMACS cDNA Synthesis Kit using the MultiMACS M96thermo Separator. The quantitative amplification of the housekeeping gene GAPDH was performed (A). Mean cycle threshold: 12; coefficient of variation 2.0%. Display of the dissociation curves of all 96 samples shows pure peaks (B).

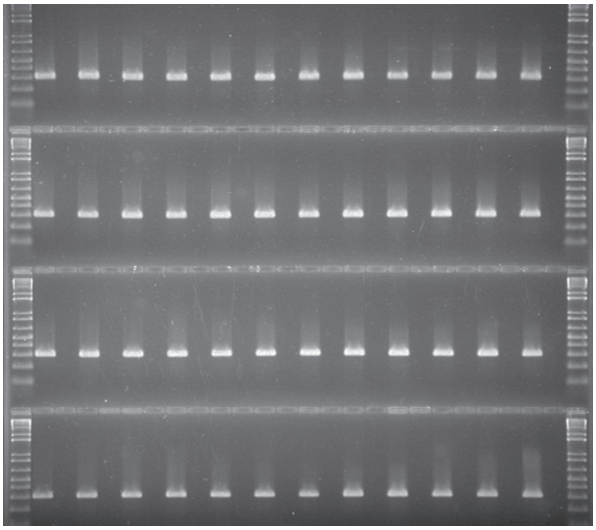


Fig. 2: Analysis of cDNA synthesized with the MultiMACS System
The MultiMACS cDNA Synthesis Kit and the MultiMACS M96thermo Separator were used to produce PCR templates, set up in a chessboard pattern: 5 µg of Molt-4 total RNA alternated with negative controls containing Lysis/Binding Buffer. PCR products were generated from 1 µL of eluate using GAPDH primers (35 cycles) and then separated by gel electrophoresis in a 2% agarose gel. The first row shows MultiMACS M96thermo 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. Molecular weight markers are in the first and last lane of every row.

1.3 Kit capacities

The MultiMACS cDNA Synthesis Kits are for isolation of mRNA from a maximum of 10^7 cells, 30 mg human and animal tissue (spleen: 10 mg, thymus: 5 mg), or 200 µg total RNA. For stabilized whole blood samples, refer to the respective special protocol at www.miltenyibiotec.com/protocols.

10^7 cells or 30 mg human or animal tissue typically yield 1–6 µg mRNA. Some quiescent cells may contain significantly lower amounts of mRNA. From cells to cDNA 96 samples can be processed in less than 120 minutes.

1.4 Reagent and instrument requirements

▲ All additionally required equipment must be RNase-free.

- Phosphate-buffered saline (PBS)
- MultiMACS M96thermo Separator
- Further microtiter plates or 8-well strips with caps

1.4.1 For homogenization and lysis

Up to 10^4 cells in a microtiter plate

- Microplate shaker

Whole blood, tissue, and more than 10^4 cells

- Multi-96 Filter or Multi-8 Filter plus Frame
- gentleMACS™ Dissociator for gentle, automated tissue dissociation. Protocol for mRNA isolation available: www.miltenyibiotec.com/protocols

  Alternatively: Bead mill, for example, Mini-Bead Beater-96, BioSpec Products, or TissueLyser, Qiagen. Stainless steel beads for parallel lysis/homogenization (refer to supplier of bead mill)
- For tissue homogenization: centrifuge compatible with tubes containing lysate (e.g. 2-mL tubes or 8-well strips)

1.4.2 For manual use

- 8-channel pipette with tips, for example, 8-Channel Impact® Pipettor from Matrix Technologies:
  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 elution buffer in a single pipetting mode
- Six disposable reagent reservoirs for multichannel pipettes as reservoirs for Lysis/Binding Buffer, Wash Buffer, Equilibration-/Wash Buffer, Resuspension Buffer, Release Solution and cDNA Elution Buffer

1.4.3 For automated use

- Liquid handling platform with four to eight pipetting channels, range 10 µL to 1 mL, and a gripper tool to grip plates sideways
- MultiMACS Adapter Plate and command line interface mumcli.exe (please contact your local Miltenyi Biotec Technical Support)
- Reservoir holder and reagent reservoirs for Lysis/Binding Buffer, Wash Buffer, MicroBeads, Equilibration-/Wash Buffer, Resuspenion Buffer, Release Solution, and cDNA Elution Buffer

1.5 Related products

- MultiMACS M96thermo Separator (# 130-094-534)
- Multi-8 Columns (# 130-092-444)
- Multi-96 Columns (# 130-092-445)
- Multi-96 Filter (# 130-092-547)
- Multi-8 Filter and Frame (# 130-092-548)
- Deep Well Block (DWB, 2.5 mL, # 130-092-549)
- PrepProtect™ Stabilization Buffer (100 mL, # 130-092-642; 10 mL, # 130-092-643), for details see chapter 3, Tips & hints
- MACS Products for cell separation: www.miltenyibiotec.com
- gentleMACS Dissociator (# 130-093-235) for gentle, automated tissue dissociation

2. Protocols for mRNA isolation and cDNA synthesis

The MultiMACS M96thermo Separator provides a list of pre-defined separation programs. Refer to the instrument manual for a program overview, and for updates please visit www.miltenyibiotec.com.

96 CDNA SYNTH
The standard program for cDNA synthesis with Multi-8 or Multi-96 Columns is called 96 CDNA SYNTH.

96 CDNA SYNTH+DNASE
The program 96 CDNA SYNTH+DNASE is installed for the rare case a DNase digestion step needs to be performed. Please refer to section 5.1, In-column removal of DNA traces using DNase I.

▲ **Caution: Read the MultiMACS M96thermo 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 M96thermo Magnet, the MultiMACS Column Holder, and the plates are in the same orientation. For details refer to the MultiMACS M96thermo Separator user manual.

▲ 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 instrument manual.

▲ All additionally required equipment must be RNase-free.

Before starting

▲ Adjust all buffers to room temperature.

▲ If purifying mRNA from tissues or more than 10^4 cells, use Multi-8 Filter for up to 48 samples and Multi-96 Filter for more than 48 samples. The filters are not included in the kit, refer to 1.4 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. For details refer to the MultiMACS M96thermo 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!

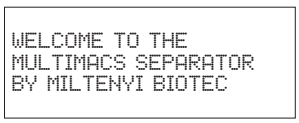
▲ Unpack the necessary amount of individual, sterile-packed Multi-8 Columns and put them in the MultiColumn Frame. Use the same pattern as for the Multi-8 Filter. Avoid touching the column tips.

For purification of 96 samples

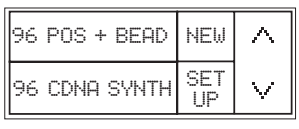
▲ Unpack Multi-96 Columns. Avoid touching the column tips.

2.1 Preparing the MultiMACS™ M96thermo Separator

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

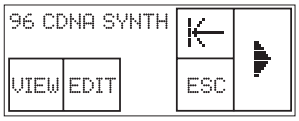


2. The last program—also referred to as process—performed on the MultiMACS M96thermo Separator is displayed on the upper left segment. The one but last process is listed below. If necessary, scroll through the list of available process names by touching the symbols ^ or v until 96 CDNA SYNTH is displayed. Touch 96 CDNA SYNTH to go to the Process Management Screen.



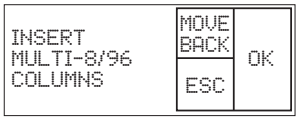
▲ **Note:** In case a DNase digestion step needs to be performed, please refer to corresponding protocol section 5.1, In-column removal of DNA traces using DNAse I.
▲ **Note:** The scroll function is only visible if there are more programs to choose from.

3. If necessary, check the process parameters (see MultiMACS M96thermo Separator User Manual). Touch ▶ to start the process and to move the magnet to the start position.

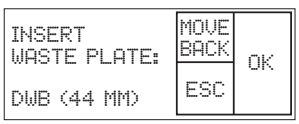


▲ **Note:** In case you want to leave the process, press ESC. If you need to go back to the previous process level, press |←.

4. Follow the instructions given on the Touch Display. Insert a MultiColumn Frame with up to twelve Multi-8 Columns or a pre-packed Multi-96 Column into the MultiMACS Column Holder.

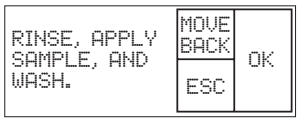


5. Touch **OK** to move the magnet upwards. The following screen appears:



6. Place a waste plate, for example, the 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 M96thermo Separator user manual).

   Touch **OK** to move the MultiMACS M96thermo Magnet downwards. Column tips now slightly immerse in the waste plate. The next screen appears.



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

8. Proceed with section 2.2.

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2.2 Sample preparation and magnetic labeling

The MultiMACS cDNA Synthesis Kits are 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 (section 2.2.3). For mRNA isolation/cDNA synthesis from whole blood, please refer to the respective special protocol on the website www.miltenyibiotec.com/protocols.

2.2.1 Lysis and magnetic labeling of cells

2.2.1.1 Lysis and magnetic labeling of suspension cells

	Filter	DNA shearing	Volume of Lysis Buffer per column (µL)
Up to 10^4 cells	n.r.	n.r.	200
10^4–5×10^5 cells	Filter **or** shearing		1000
5×10^5–5×10^6 cells	n.r.	required	1000
5×10^6–10^7 cells	required	required	1000

Table 1: Overview of requirements for lysis
Depending on cell numbers, the required filtering steps, DNA shearing steps, and corresponding amounts of Lysis Buffer are listed.
n.r.: not required

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 minutes at 300×g and aspirate the entire supernatant.
2. Premix 30 µL of Oligo(dT) MicroBeads and 200 µL of Lysis/Binding Buffer per well. Add mixture (230 µL) to each well containing a cell pellet, and shake at 750 rpm for 5 minutes 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 within 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 Filters in a Multi-8 Filter Frame, which are not supplied with the kit, and (optionally) a Deep Well Block (2.5 mL) are used.

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

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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 minutes at 300×g and aspirate the supernatant completely. Resuspend cell pellet by vortexing it shortly.
3. Premix 30 µL of Oligo(dT) MicroBeads and 1000 µL of 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 minutes.
5. Apply 200 µL of 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 within 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 Filters 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 minutes at 300×g and aspirate supernatant completely.
2. Premix 30 µL of Oligo(dT) MicroBeads and 1000 µL of 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 minutes. 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 within the columns.

   For 5×10^6–10^7 cells
   Unpack and place a Multi-96 Filter or up to six Multi-8 Filters 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 within the Multi-8/96 Columns. Apply 200 µL of 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 of Oligo(dT) MicroBeads and 200 µL of Lysis/Binding Buffer per well. Add mixture (230 µL) to each well containing cells and shake at 750 rpm for 5 minutes 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 within the columns.
4. Proceed with section 2.3.

Up to 10^7 cells in other culture formats

▲ It is recommended to detach cells, for example, 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 minutes 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 Buffer (see chapter 3, Tips & hints). Samples can be lysed individually with a rotor-stator homogenizer or in parallel with a bead mill described in detail below.

1. For samples stabilized in PrepProtect Buffer: First, remove PrepProtect Buffer completely.
2. Transfer samples into 8-well strips. Put two stainless steel beads (diameter 5 mm) in each well, add 900 µL of 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 minutes. Use a maximum of 30 mg of tissue (spleen: 10 mg, thymus: 5 mg). For many tissues, 30 mg corresponds to a piece of approximately 3 mm × 3 mm × 3 mm (except, e.g., lung, fat, or skin).

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

4. Centrifuge 8-well strips for 3 minutes at 6000×g. Prepare a 96-well plate by pipetting 30 µL of 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:** 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 minutes 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 of 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 of 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 minutes 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 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 of 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 within the Multi-8 Columns.
4. Proceed with step 2.3.

2.3 Purification of mRNA

▲ To avoid foaming pipette buffers in a multidispense mode (one aspiration for all dispenses).

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

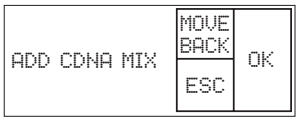
1. Rinse Multi-8/96 Columns with 2× 200 µL of Lysis/Binding Buffer. Only for total RNA samples one single rinse is sufficient.
2. Rinse Multi-8/96 Columns with 4× 100 µL of Wash Buffer.
3. Proceed with step 2.4, cDNA synthesis

▲ **Note:** If a DNase digestion step needs to be performed, please refer to section 5.1, step 8, In-column removal of DNA traces using DNase I.

2.4 cDNA synthesis

▲ Do not elute the mRNA!
cDNA synthesis will be performed directly in the Multi-8/96 Column.

1. Rinse Multi-8/96 Columns with 2× 100 µL of Equilibration/Wash Buffer. Touch **OK** and the following screen appears:



2. Dissolve the lyophilized Enzyme Mix in 20 µL of Resuspension Buffer (RB).

▲ **Note:** It is not necessary to pipette the Enzyme Mix up and down more than twice. The lyophilized mix will be dissolved in a few minutes.

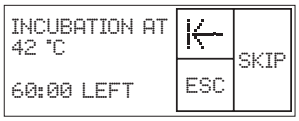
3. Apply 20 µL of resuspended Enzyme Mix on top of the column matrix.
4. Touch **OK** and the following screen appears.



5. Move the Tip-Touch Plate firmly back and forth once so that the tips of the Multi-8 Columns touch the inner walls of the Deep Well Block. Thereby, any drop on the column tips that did not fall off by gravity will be removed.
6. Touch **OK** to heat the MultiMACS M96thermo Magnet to 42 °C.



The next screen appears. It shows the remaining incubation time running down for a total of 1 hour.

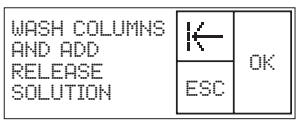


7. When the incubation time is over, an acoustic signal resounds and overtime will be counting up until the next step will be demanded.

▲ **Note:** Extending the specified incubation time may not lead to higher cDNA yield. In contrast, extreme overtime can degrade cDNA or clog the column by evaporation.



8. Touch **OK** to stop the acoustic alarm and the following screen appears:



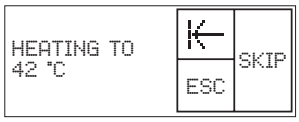
2.5 Purification and elution of cDNA

1. Rinse column with 2×100 µL of Equilibration/Wash Buffer.
2. Apply 20 µL of cDNA Release Solution on top of the column matrix.
3. Touch **OK** and the following screen appears:



4. 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.

   Touch **OK** to heat the MultiMACS M96thermo Magnet to 42 °C.



5. The next screen appears. It shows the remaining incubation time running down for a total of 10 minutes.



2. cDNA synthesis

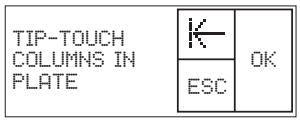
6. When the incubation time is over, an acoustic signal resounds and overtime will be counting up until the next step will be demanded.



7. Touch **OK** to stop the acoustic alarm and the following screen appears:

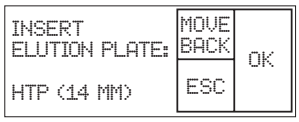


8. Apply 10 µL of cDNA Elution Buffer (EB) on top of the column matrix.
9. Touch **OK** and the following screen appears:

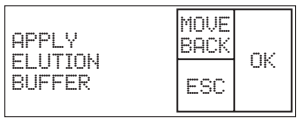


10. Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the Deep Well Block touch the tips of the Multi-8 Columns. Thereby, any drop on the column tips that did not fall off by gravity will be removed.

11. Touch **OK** to move the MultiMACS M96thermo Magnet upwards. The next screen appears.



12. Remove Deep Well Block. Insert the elution plate (Microtiter Plate).
13. Touch **OK** and the MultiMACS M96thermo Magnet will move downwards until column tips slightly immerse in the elution plate. The following screen appears:

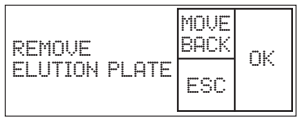


14. Apply 30 µL of cDNA Elution Buffer (EB) directly onto the Multi-8/96 Column matrix. Let the buffer pass through.
15. Touch **OK** and the next screen appears.

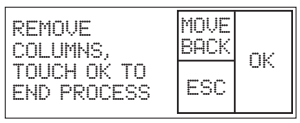


2. cDNA synthesis

16. 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.
17. Touch **OK** to move the MultiMACS M96thermo Magnet upwards.



18. Remove Elution Plate. The cDNA 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.
19. Touch **OK** to move the MultiMACS M96thermo Magnet away from the Column Holder.



20. Remove MultiColumn Frame with Multi-8/96 Columns. Touch **OK** to finish the process.

3. Tips & hints

Removal of enzyme mix plate sealing

The plastic film sealing of the enzyme mix plate can either be peeled off by hand or pierced with a pipette tip. In the case of piercing, we recommend wiping the foil with an RNase removing solution such as RNaseZap® (Ambion) to reduce the risk of contaminating the cDNA synthesis reaction with RNases. To pierce the plastic film, use a fresh pipette tip; to resuspend the Enzyme Mix, a new pipette tip should be used.

Quantitative PCR

For quantitative PCR reactions (e.g. TaqMan®), we recommend using a maximum of 10–20% of the cDNA solution to obtain best results. The total amount of cDNA typically used should not exceed 1 ng; for more specific recommendations, please consult the manufacturer's manual of the quantitative PCR device.

Residual mRNA after cDNA synthesis

In rare cases, PCR reactions may fail due to residual mRNA bound to cDNA. In this primer-dependent process, we recommend using either RNase H to digest mRNA before the PCR reaction or choosing another pair of primers.

For RNAse H digestion, add 2 units of RNAse H in 20 µL of 1× RNase H Buffer after washing the column (section 2.5, step 1) and incubate for 5 minutes at 37 °C in the MultiMACS M96thermo Separator. Rinse column with 2×100 µL of Equilibration/Wash Buffer and proceed with the cDNA Release Solution (section 2.5, step 2).

4. Troubleshooting

4.1 Low cDNA yield

Scarce mRNA source

The amount of poly(A) RNA depends on sample type and physiological state (1–5% of total RNA). 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, for example, 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 tissues that were stabilized in PrepProtect Buffer prior to freezing, heat these lysates for 5 minutes at 70 °C and allow to cool to room temperature for another 10 minutes.

Degraded RNA

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

4.2 Avoid mRNA degradation

Sample collection and storage

After sample collection, work quickly until samples are completely lysed, quick-frozen in liquid nitrogen, or stabilized in PrepProtect Buffer. Do not let frozen tissue thaw unless it is stabilized in PrepProtect Buffer. To ensure a good penetration by PrepProtect Buffer, 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, for example, Disposable Reagent Reservoirs from Matrix Technologies Corp., are recommended.

cDNA analysis

Use a positive control to rule out problems with the analysis procedure, for example, RNA molecular weight marker with poly(A) tail or a RNA sample that is known to be intact.
It could also be helpful to check the synthesized cDNA in a PCR with a housekeeping gene.

4.3 Variation in cDNA 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.
Always be sure that the applied volumes, especially the small ones, are pipetted directly onto the column matrix and run into the columns.
Do not exceed the given incubation time, as this may lead to evaporation of the column volume.

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.

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, for example, 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.

During mRNA isolation and cDNA synthesis

Before moving the MultiMACS M96thermo Magnet, for example, to change the Deep Well Block, make a Tip-Touch to remove any residual drop at the column tip.

4.5 Clogging of column or 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.
If the lysis is difficult to perform, reduce the amount of starting material.

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 may not be completely removed. The MultiMACS mRNA/cDNA isolation technology has been 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 remove contaminating genomic DNA, a short DNase I treatment directly in the column following the MultiMACS mRNA isolation can be performed. No inactivation or precipitation step is required as the DNase I is simply washed away from the column.

▲ **Note:** However, for standard applications, we do not recommend DNase digestion since DNase solutions may contain RNases. If a DNase digestion is necessary, the recommended incubation time is 20 minutes at room temperature (please refer to the manufacturer's recommendations). If required, edit the program and change incubation time and temperature.

5.1.1 Reagent and instrument requirements

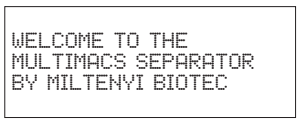
- RNase-free DNase I (e.g. Ambion, # 2222, 2 U/µL)
  2 U (1 µL) DNase I / reaction is required
- DNase buffer, RNase-free, supplied with DNase I; or prepare standard DNase I buffer, for example:
  10× DNase I Buffer: 100 mM Tris HCl (pH 7.5)
  25 mM $MgCl_2$
  5 mM $CaCl_2$

Necessary volumes of 1× DNase I Buffer/reaction: 120 µL.

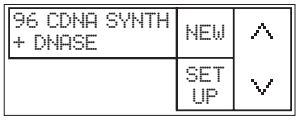
5.1.2 Protocol for in-column DNase I treatment

Select 96 CDNA SYNTH+DNASE

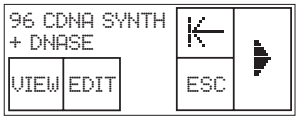
1. In case you need to perform a DNase digestion step, directly choose the special process termed 96 CDNA SYNTH+DNASE. After switching on the instrument, touch the Welcome Screen or wait for a few seconds until the Process Selection Screen appears.



2. The last process performed on the MultiMACS M96thermo Separator is displayed on the upper left segment. The one but last process is listed below. If necessary, scroll through the list of available process names by touching the symbolss ^ or v until 96 CNDA SYNTH+DNASE is displayed. Touch 96 CDNA SYNTH + DNASE to go to the Process Management Screen.

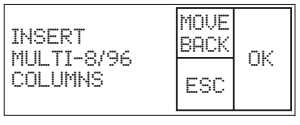


▲ **Note:** The scroll function is only visible if there are more programs to choose from.



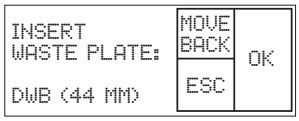
▲ **Note:** In case you want to leave the process, press ESC. If you need to go back to the previous process level, press |←.

3. If necessary, check the process parameters (see MultiMACS M96thermo 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.



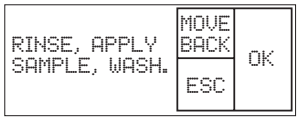
Prepare columns

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 a waste plate, for example, the 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 M96thermo Separator user manual).

   Touch **OK** to move the MultiMACS M96thermo Magnet downwards. Column tips now slightly immerse in the waste plate. The next screen appears.



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

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Sample preparation and magnetic labeling

7. Proceed with section 2.2, Sample preparation and magnetic labeling, and section 2.3, Purification of mRNA.

   ▲ **Note:** After completion of section 2.3, step 2 (rinsing Multi-8/96 Columns with 4×100 µL of Wash Buffer), the process 96 CDNA SYNTH+DNASE for in-column removal of DNA traces using DNase I diverges from the process 96 CDNA SYNTH. Therefore, please follow the protocol as detailed below starting with step 8.

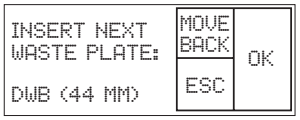
Removal of DNA traces

8. Touch **OK** and the following screen appears:



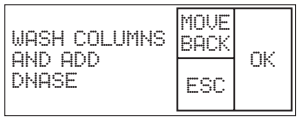
   Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the Deep Well Block touch the tips of the Multi-8 Columns. Thereby, any drop on the column tips that did not fall off by gravity will be removed.

9. Touch **OK** to move the MultiMACS M96thermo Magnet upwards. The next screen appears.



10. Place a new waste plate, for example, the 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 M96thermo Separator user manual).

11. Touch **OK** to move the MultiMACS M96thermo Magnet downwards. Column tips now slightly immerse in the waste plate. The next screen appears.



12. Wash columns with 1×100 µL of DNase I Buffer per column.

13. Prepare DNase I reaction solution: Add 1 µL of DNase I in 20 µL of 1× DNase I Buffer per column. Apply DNase I reaction solution onto columns.

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



15. 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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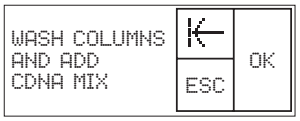
16. Touch **OK** to start the incubation time. The next screen appears. It shows the remaining incubation time running down for a total of 20 minutes.



17. When the incubation time is over, an acoustic signal resounds and overtime will be counting up until the next step will be demanded.



18. Touch **OK** to stop the acoustic alarm and the following screen appears:



19. Wash columns with Lysis/Binding Buffer: 2×200 µL per column.

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

21. Rinse Multi-8/Multi-96 Columns with 2×100 µL of Equilibration/ Wash Buffer.

cDNA synthesis

22. Dissolve the lyophilized Enzyme Mix in 20 µL of Resuspension Buffer (RB) per column.

    ▲ **Note:** It is not necessary to pipette the Enzyme Mix up and down more than twice. The lyophilized mix will be dissolved in a few minutes.

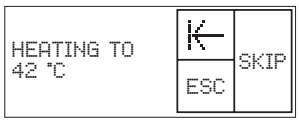
23. Apply 20 µL of resuspended Enzyme Mix on top of each column matrix.

24. Touch **OK** and the following screen appears:

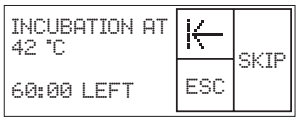


25. Move the Tip-Touch Plate firmly back and forth once so that the tips of the Multi-8 Columns touch the inner walls of the Deep Well Block. Thereby, any drop on the column tips that did not fall off by gravity will be removed.

26. Touch **OK** to heat the MultiMACS M96thermo Magnet to 42 °C.



The next screen appears. It shows the remaining incubation time running down for a total of 1 hour.

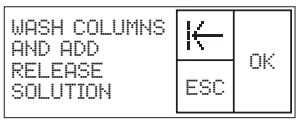


27. When the incubation time is over, an acoustic signal resounds and overtime will be counting up until the next step will be demanded.

    ▲ **Note:** Extending the specified incubation time may not lead to higher cDNA yield. Rather extremely overtime can degrade cDNA or clog the column by evaporation.



28. Touch **OK** to stop the acoustic alarm and the following screen appears:



29. Proceed with section 2.5, Purification and elution of cDNA.

5.2 Buffer amounts

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

No. of samples	Micro-Beads (mL)	Lysis/ Binding Buffer[1] (mL)	Lysis/ Binding Buffer[2] (mL)	Lysis/ Binding Buffer[3] (mL)	Wash Buffer (mL)	Equi-libration-Wash Buffer (mL)	Resuspen-sion Buffer (mL)	Release Solution (mL)	Elution Buffer (mL)
8	0.24	11.2	12.8	4.8	3.2	3.2	0.16	0.16	0.32
16	0.48	22.4	25.6	9.6	6.4	6.4	0.32	0.32	0.64
24	0.72	33.6	38.4	14.4	9.6	9.6	0.48	0.48	0.96
32	0.96	44.8	51.2	19.2	12.8	12.8	0.64	0.64	1.6
40	1.2	56.0	64.0	24.0	16.0	16.0	0.8	0.8	1.92
48	1.44	67.2	76.8	28.8	19.2	19.2	0.96	0.96	1.92
56	1.68	78.4	89.6	33.6	22.4	22.4	1.12	1.12	2.24
64	1.92	89.6	102.4	38.4	25.6	25.6	1.28	1.28	2.56
72	2.16	100.8	115.2	43.2	28.8	28.8	1.44	1.44	2.88
80	2.4	112.0	128.0	48.0	32.0	32.0	1.6	1.6	3.2
88	2.64	123.2	140.8	52.8	35.2	35.2	1.76	1.76	3.52
96	2.88	134.4	153.6	57.6	38.4	38.4	1.92	1.92	3.84

Table 2: Required buffer volumes

1) Sample lysed in 1 mL, no Multi-8/96 Filter is used.
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 is used.

6. References

1. Mack, E. *et al.* (2007) Comparison of RNA yield from small cell populations sorted by flow cytometry applying different isolation procedures. Cytometry A. 71: 404–409.

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