MACS molecular

Short protocol

µMACS™ mRNA Isolation Kit

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

µMACS™ mRNA Isolation Kits – Short protocol

For information on sample material and volume, homogenization and lysis procedure, please refer to the detailed protocol with tips and hints in the µMACS™ mRNA Isolation Kits data sheet.

Before starting: Warm up Elution Buffer to 70 °C using a heating block.
Warm up Lysis / Binding Buffer and Wash Buffer to room temperature.

1.1 Sample preparation from cells, tissue, or whole blood

1. Homogenize and lyse cells, tissue, or whole blood according to user manual. Perform DNA shearing step when using tissue, whole blood, or >5×10^6 cells.

	Adherent or suspension cells	Human or animal tissue	Plant tissue	Whole blood
Small Scale (µ Column)	up to 10^7	up to 30 mg	up to 100 mg	up to 0.5 mL
Addition of Lysis/ Binding Buffer	1 mL	1 mL	1 mL	1 mL (final volume)
Large Scale (M Column)	up to 5×10^7	up to 150 mg	up to 500 mg	up to 2.5 mL
Addition of Lysis/ Binding Buffer	1–5 mL (1 mL per 10^7)	5 mL	5 mL	5 mL (final volume)

▲ **Note:** Incomplete lysis and high viscosity will slow down column flow and affect mRNA yield. Check that no fuzzy material or clumps remain in the lysate. In case of fuzzy material or viscosity insert an additional DNA shearing step. For details, please refer to µMACS mRNA Isolation Kits data sheet.

2. Apply lysate on top of the LysateClear Column that is placed in the centrifugation tube. LysateClear Columns remove cell debris while the cleared lysate is collected in the centrifugation tube.

   Small scale LysateClear Column: centrifuge at ≥ 13,000×g for 3 minutes.

   Large scale LysateClear Column: centrifuge at ≥ 5,000×g for 10 minutes.

1.2 Sample preparation from total RNA

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

Small Scale: use up to 200 µg total RNA (maximum volume: 500 µL)
Large Scale (Total RNA Kit): use up to 1 mg total RNA (maximum volume: 2.5 mL)

1. Heat total RNA for 5 minutes at 70 °C. Then, chill briefly on ice. 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 minimum sample volume of 250 µL (µ Column) or 1.25 mL (M Column).

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2. Magnetic labeling and isolation

1. Place a MACS® Column in the magnetic field of an appropriate MACS Separator. Prepare column by rinsing with **Lysis/Binding Buffer**.
   µ Column: 100 µL
   M Column: 250 µL

2. Add **Oligo (dT) MicroBeads** to the prepared sample and mix by pipetting up and down 2–3 times or by short vortexing.
   For cells, tissues, and whole blood: 50 µL per 1 mL lysate (prepared in section 1.1).
   For total RNA: 25 µL Oligo (dT) MicroBeads per diluted 100 µg total RNA (prepared in section 1.2). For less total RNA, also use 25 µL.
   ▲ **Note:** For the hybridization of mRNA to Oligo (dT) MicroBeads, further incubation is not necessary.

3. Apply lysate on top of the column matrix. Magnetically labeled mRNA is retained in the column.

4. Rinse column with **Lysis/Binding Buffe**r to remove proteins and DNA.
   µ Column: 2×200 µL (total RNA sample: 1×200 µL)
   M Column: 3×250 µL (total RNA sample: 1×250 µL)

5. Rinse column with **Wash Buffer** to remove rRNA and DNA.
   µ Column: 4×100 µL
   M Column: 4×250 µL

6. Pre-elution: Apply pre-heated (70 °C) **Elution Buffer** using 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. Reuse of one pipet tip for multiple pipetting steps with hot buffer can raise 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.

7. Elution: Place a new RNase-free tube beneath the column.
   ▲ **Note:** For elution of mRNA, the column should remain in the magnetic field.

   Apply pre-heated **Elution Buffer**.
   µ Column: 50 µL
   M Column: 75 µL

   Alternative elution: To increase mRNA yield up to 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.
   ▲ **Note:** Collect 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.