MACS molecular

Isolation of mRNA from yeast cells

µMACS™ mRNA Isolation Kits 130-075-201
130-090-276

The following protocol describes the isolation of mRNA from yeast cells (*Schizosaccharomyces pombe*). The yeast cell wall is enzymatically removed using zymolyase. Yeast spheroplasts are then lysed using Lysis/Binding Buffer. The viscosity of the lysate is reduced by centrifuging with a LysateClear Column. For magnetic labeling of the poly-A$^+$ mRNA, Oligo (dT) MicroBeads are added to the lysate and the mRNA is selected on a µ Column. The µMACS™ mRNA Isolation Kit offers a rapid and convenient protocol for the isolation of highly pure, functional yeast mRNA in only one step.

1. Instrument and reagent requirements

- µMACS mRNA Isolation Kit (# 130-075-201).
- µMACS or thermoMACS™ Separator.
- RNase-free tubes and pipette tips.
- Heat block placed close to the µMACS Separator and pre-heated to 70 °C.
- Microcentrifuge suitable for 2 mL tubes.
- YE medium (3% glucose; 0.5% yeast extract).
- Protoplasting solution (0.9 M sorbit; 5 mM EDTA, pH 4.9 supplemented with 2 mg/mL zymolyase).

2. Growth of yeast cells

1. Two days before the experiment, inoculate 20 mL YE medium with a single yeast colony of the strain to be analyzed. Grow for 2 days at 28 °C with constant shaking.
2. The night before mRNA isolation, inoculate 100 mL YE medium with an appropriate amount of the yeast pre-culture (e.g. 1 mL, depending on the yeast strain used). Grow overnight to a titer of 8×10^7 cells/mL.
3. Centrifuge cells for 5 minutes at 1,100×g at room temperature.
4. Resuspend cell pellet in 5 mL sterile, distilled water.
5. Repeat centrifugation as described above and resuspend cell pellet in 5 mL sterile, distilled water.
6. Determine the cell titer.

3. Preparation of spheroplasts

1. Centrifuge approximately 1×10^9 cells in a 1.5 mL tube at 1,100×g for 5 minutes at room temperature.
2. Resuspend the cell pellet in 0.5 mL protoplasting solution and incubate at 37 °C.
3. After approximately 15 minutes, most of the cell wall should be removed. Check if conversion to spheroplasts has been completed by observing the degree of spheroplast lysis in water under a microscope. Spheroplasts will lyse in a nonisotonic medium such as water whereas intact yeast cells will not.

4. Isolation of mRNA from yeast spheroplasts

Before starting: Warm up Elution Buffer to 70 °C.

1. Centrifuge approximately 2×10^8 spheroplasts contained in a 1.5 mL tube at 1,000 rpm in a microcentrifuge for 5 minutes. Remove the supernatant and add 1 mL of Lysis/Binding Buffer. Lyse cells completely by vigorous vortexing for about 3 minutes.
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. Centrifuge at 13,000×g for 3 minutes.
3. Place a **MACS® µ Column** in the magnetic field of the µMACS Separator.
4. Apply 100 µL of **Lysis/Binding Buffer** and let buffer run through.
   ▲ Note: Columns are "flow stop" and do not run dry.
5. Add 50 µL Oligo (dT) MicroBeads to the prepared sample and mix by pipetting up and down 2–3 times or by short vortexing.
   ▲ Note: For the hybridization of mRNA to Oligo (dT) MicroBeads, further incubation is not necessary.
6. Apply lysate on top of the column matrix. Let the lysate pass through. Magnetically labeled mRNA is retained in the column.
7. Rinse column with 2×200 µL of **Lysis/Binding Buffer** to remove proteins and DNA.
8. Rinse column with 4×100 µL of **Wash Buffer** to remove rRNA and DNA.
9. Pre-elution: Apply 27 µL pre-heated (70 °C) **Elution Buffer** using a fresh pipet tip for each pepetting step. Discard flow-through.
   ▲ **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.
10. Elution: Place a new RNase-free tube beneath the column.
    ▲ **Note:** For elution of mRNA, the column should remain in the magnetic field.

    Apply 50 µL pre-heated **Elution Buffer**.

    Alternative elution: To increase mRNA yield up to 10%, apply 75 µL pre-heated **Elution Buffer**.

    ▲ **Note:** The alternative elution will increase the volume of the eluate an decease 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.

SP0072.05

Miltenyi Biotec

www.miltenyibiotec.com

Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925