Miltenyi Biotec

Homogenization of tissue for mRNA isolation

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1. Description

1.1 Background information

The isolation of subcellular material such as mRNA from tissues or cells requires fast and thorough homogenization of the respective starting material. The gentleMACS™ Dissociators provide optimized programs that meet these requirements. In combination with M Tubes, the gentleMACS Dissociators allow the automated homogenization of tissues in a closed system, enabling sterile sample handling. A single sample or two samples can be processed in parallel.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator (# 130-093-235)
- gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS M Tubes (# 130-093-236, # 130-096-335)
- µMACS™ mRNA Isolation Kits: µMACS mRNA Isolation Kit-Small Scale (# 130-075-201), µMACS mRNA Isolation Kit-Small Scale (# 130-090-276), µMACS mRNA Starting Kit (# 130-075-202), µMACS mRNA Isolation Kit-Large Scale (# 130-075-101), µMACS mRNA Isolation Kit-Large Scale (# 130-090-277)
- (Optional) Antifoam Y-30 emulsion (e.g. Sigma-Aldrich, A6457)

2. Protocol for homogenization of tissue for mRNA isolation

▲ The protocol has been tested successfully for a range of mouse tissues, such as liver, lung, brain, spleen, kidney, or heart.

▲ If working with fibrous and/or RNase-rich material, such as mouse tail, ear, skin, muscle, or pancreas it is required to prepare total RNA prior to mRNA isolation. Please refer to the gentleMACS Protocol "Homogenization of tissue for total RNA isolation".

▲ **Note:** Very hard material such as bone should not be processed since it may damage the M Tubes.

▲ Sample volumes of 1 mL or 5 mL are processed per M Tube. For details, please refer to µMACS mRNA Isolation Kit data sheets.

▲ Its molecular characteristics make RNA chemically unstable and inherently susceptible to ubiquitous RNases. It is therefore recommended to rapidly lyse samples in Lysis/Binding buffer without interruptions to minimize mRNA degradation. Avoid thawing of frozen samples before lysis.

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Choose one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
2. Adjust Lysis/Binding Buffer of µMACS mRNA isolation kit to room temperature.
3. Add Antifoam A to a final concentration of 0.5% to the Lysis/Binding Buffer to prevent excessive foam formation during sample homogenization.
4. Pipette Lysis/Binding Buffer with 0.5 % Antifoam into the M Tube: 1 mL (small scale kit) or 5 mL (large scale kit). For details refer to the µMACS mRNA isolation kit data sheets.
5. Transfer tissue sample into the Lysis/Binding Buffer in the M Tube.
   ▲ **Note:** Place sample directly into the buffer to avoid adherence of the tissue to the tube wall.
6. Tightly close M Tube and turn the tube upside down in one quick move ensuring that the sample material reaches the area of the rotor/stator.
7. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
8. Run one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
9. After termination of the program, detach M Tube from the gentleMACS Dissociator.
10. Centrifuge sample at 2000×g for one minute.
11. Remove the homogenized sample from the tube.
    ▲ **Note:** Homogenized tissue can be removed from the closed M Tube by pipetting through the septum-sealed opening in the center of the cap of the M Tube. Use ART 1000 REACH 1000 µL pipette tips.
12. Proceed with mRNA isolation as described in the µMACS mRNA Isolation Kit data sheets.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

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Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com