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1. Description

1.1 Background information

The isolation of subcellular material such as proteins 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.

This protocol has been developed for the homogenization of tissues to extract cytoplasmic proteins for subsequent analysis such as Western blotting.

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)
- Appropriate lysis buffer for the protein of interest
- (Optional) Antifoam Y-30 emulsion (e.g. Sigma-Aldrich®, # A6457)

2. Protocol for homogenization of tissue for protein extraction

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

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

▲ The sample volume should be between 300 µL and a maximum of 10 mL of lysis buffer. It is recommended to use up to 10 mg tissue per mL of lysis buffer.

▲ Pre-cool lysis buffer on ice.

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

1. Choose the gentleMACS Program **Protein_01**.
2. Pipette appropriate amount of ice-cold lysis buffer into the M Tube.
3. (Optional) To avoid excessive foam formation during sample homogenization, add Antifoam Y-30 to a final concentration of 1% to the lysis buffer.
4. Transfer tissue sample into the lysis buffer in the M Tube.
▲ **Note:** Place sample directly into the buffer to avoid adherence of the tissue to the tube wall.
5. 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.
6. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator.
7. Run the gentleMACS Program **Protein_01**.
8. After termination of the program, detach M Tube from the gentleMACS Dissociator.
9. Centrifuge sample at 4000×g for 5 minutes to collect lysate at the tube bottom.
10. Store on ice until proceeding with protein analysis.

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

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