Homogenization of tissue for total RNA isolation

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1. Description

1.1 Background information

The isolation of subcellular material such as total RNA 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)
- Total RNA isolation kits from different suppliers

2. Protocol for homogenization of tissue for total RNA isolation

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

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

▲ The sample volume should be between min. 350 µL and max. 10 mL of lysis buffer.

▲ 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 RNA 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 buffer to room temperature.
3. According to the kit manufacturer's recommendations pipette an appropriate amount of lysis buffer provided by the total RNA isolation kit into the M Tube.
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 it upside down onto the sleeve of the gentleMACS Dissociator.
7. Run one of the following gentleMACS Programs:
   For fresh tissue: gentleMACS Program **RNA_01**
   For frozen tissue: gentleMACS Program **RNA_02**
8. After termination of the program, detach M Tube from the gentleMACS Dissociator.
9. (Optional) For sample volumes below 3 mL or if excessive foam formation occurred during the homogenization process, centrifuge M Tube at 2000×g for 1 minute to collect lysate at the tube bottom.
10. 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.
11. Proceed with total RNA isolation according to the kit manufacturer's recommendations.

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

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