Isolation of mRNA from stabilized whole blood

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1. Description

1.1 Background

High-quality RNA is a prerequisite for many gene expression profiling techniques such as quantitative PCR or microarray analysis. However, RNA is chemically unstable and susceptible to ubiquitous RNases. RNase-associated degradation during sample collection, storage, thawing of frozen samples, and RNA preparation may jeopardize downstream analysis. This is especially true when preparing RNA from blood samples. Efficient isolation of intact RNA from blood is often hampered by the long delay between collection and RNA isolation, as well as the low amount of RNA in blood cells. Expression profiling on whole blood is also made difficult by the variable composition of the blood cell types. In addition, mRNA from whole blood mainly consists of alpha and beta globin transcripts and this leads to a significant reduction in the ability to detect low abundance transcripts. These mRNAs will generate a 700 bp peak in capillary electrophoresis, e.g. mRNA Pico assays on the Agilent Bioanalyzer 2100, Agilent Technologies.

By isolation of the specific blood cell type of interest, significance of downstream analyses, like gene expression profiling, is increased. Cell populations can be separated with MACS® Technology from PBMCs, or directly from whole blood with the autoMACS™ Separator: After incubation with MACS MicroBeads at 2–8 °C, the magnetically labeled cells are separated in a MACS Column in a fast process. Please refer to the specific data sheet of MACS Cell Separation product for further information.

When cell separation is not applicable, mRNA molecules in whole blood can be stabilized by adding a cell-lysing stabilization buffer, like PAXgene. This special protocol describes the use of PAXgene Blood RNA Tubes in combination with µMACS or MultiMACS mRNA Isolation Kits.

1.2 Research applications

Stabilization of freshly drawn blood for storage or shipment before RNA isolation.

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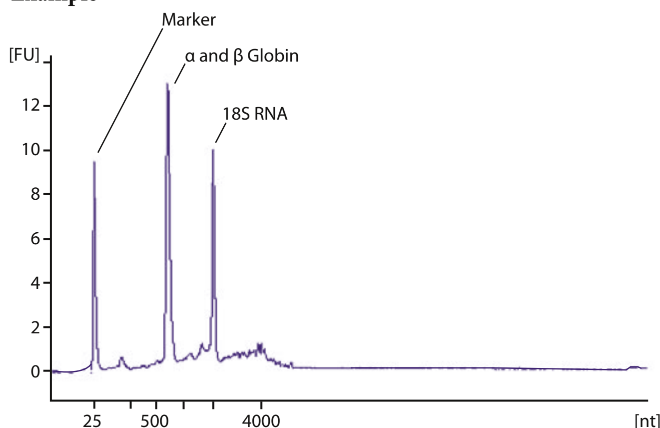
Example



In PAXgene Blood RNA Tubes 2.5 mL of whole blood was collected. After storage for one day at ambient temperature, the mRNA was purified with the µMACS™ mRNA Isolation Kit. 1 µL was analyzed with the mRNA Pico Assay on the Bioanalyzer 2100, Agilent Technologies.

1.3 µMACS™ mRNA Isolation from whole blood

µMACS™ mRNA Isolation enables a magnetic mRNA purification directly from cells with MACS Column Technology, please refer to µMACS or MultiMACS™ mRNA Isolation Kit user manuals. In combination with PAXgene Blood RNA Tubes, whole blood can be directly stabilized and then used for mRNA isolation, please read http://www.preanalytix.com/FAQ.asp for more information.

If residual genomic DNA should be removed for sensitive downstream applications, a special protocol for in-column DNase I treatment can be applied, available on www.miltenyibiotec.com.

1.4 Reagent and instrument requirements

- µMACS mRNA Isolation Kit, Small Scale, 20 reactions, # 130-075-201
- µMACS mRNA Isolation Kit, Small Scale, 10 reactions, # 130-090-276
- MultiMACS mRNA Isolation Kit, (4×96) # 130-092-519
- MultiMACS mRNA Isolation Kit (12×8) # 130-092-520

Please refer to µMACS or MultiMACS mRNA Isolation user manual for further requirements.

- (Optional) Reagents for DNase I treatment as described in special protocol, www.miltenyibiotec.com.
- PAXgene Blood RNA Tubes # 762165 (BD)
- Blood Collection Set # 367286 (BD)
- BD Vacutainer One-Use Holder # 364815 (BD)
- For µMACS mRNA Isolation: 21G needle and 1 mL syringe
- For MultiMACS mRNA Isolation: Multi-8/96 Filter and bead mill

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2. Protocol for µMACS™ or MultiMACS™ mRNA Isolation from stabilized whole blood

▲ For the protocols described below, the µMACS™ mRNA Isolation user manual or the MultiMACS™ mRNA Isolation user manual is required.

2.1 Stabilization of whole blood

1. Collect 2.5 mL of whole blood in PAXgene Blood RNA Tubes according to RNA Tube Product Circular (http://www.preanalytix.com/pdf/prodcir.pdf). Gently invert tubes 8–10 times and store them upright at room temperature for at least 2 h before storage or shipping.

2.2 Preparation of stabilized whole blood for mRNA isolation

Before starting

For frozen PAXgene Blood RNA Tubes: Thaw frozen tubes at room temperature for at least 1 h for −20 °C samples and 2 h for −70 °C samples. Samples need to reach room temperature before processing.

▲ **Note:** Do not thaw tubes at elevated temperatures in a water bath or incubator.

1. Incubate PAXgene Blood RNA Tubes for another 2 h minimum at room temperature, if this incubation was not performed immediately after collection.

   ▲ **Note:** A prolonged incubation (e.g. overnight) might slightly increase RNA yield.

2. Centrifuge the PAXgene Blood RNA Tubes containing 2.5 mL whole blood for 10 min at 3000–5000×g in a swing-out rotor.

3. Uncap and store the closure. Remove supernatant by pipetting or decanting.

4. Add 4 mL of RNase-free water to the pellet, close the tube again with the closure and vortex the pellet until it is completely dissolved.

5. Centrifuge the tube for 10 min at 3000–5000×g in a swing-out rotor. Discard the entire supernatant by pipetting or decanting. In the latter case dry the rim of the tube with a clean paper towel.

   ▲ **Note:** Incomplete removal of supernatant will dilute lysis buffer and inhibit lysis.

6. Proceed with µMACS mRNA Isolation, chapter 2.3, or MultiMACS mRNA Isolation chapter 2.4.

2.3 µMACS™ mRNA Isolation

1. Add 1mL of Lysis/Binding Buffer to the pellet and lyse pellet by intermittent vortexing for 1 min.

   ▲ **Note:** A complete lysis is extremely important for further steps.

2. To reduce viscosity of the lysate, mechanical shearing of DNA must be performed. Make sure that no fuzzy material or clumps remain in the lysate. Force the lysate at least 5 times through a 21G needle attached to a 1 mL syringe.

   ▲ **Note:** Avoid aspiration of air that will cause foaming. The foam, generated during lysis, can be reduced by centrifuging the lysate tube.

3. Place LysateClear Column, included in the µMACS™ mRNA Isolation Kit, in the centrifugation tube. Apply sheared lysate without foam on top of the LysateClear Column, Centrifuge LysateClear Column at ≥13,000xg for 3 min.

   LysateClear Columns remove cell debris while the cleared lysate is collected in the centrifugation tube.

4. Proceed with µMACS mRNA user manual, chapter 2.2 Magnetic labeling and isolation.

▲ All user manuals are available for download on the website www.miltenyibiotec.com

2.4 MultiMACS™ mRNA Isolation

1. Execute steps according to MultiMACS™ mRNA Isolation user manual up to section, 2.2 Sample preparation and magnetic labeling.

2. Add 2 mL of Lysis/Binding Buffer to the pellet and lyse pellet by intermittent vortexing for 1 min. Transfer lysate to a 2 mL tube.

3. Add 60 µL of MicroBeads to the lysate.

4. To reduce viscosity of the lysate, mechanical shearing of DNA must be performed. Add one stainless steal bead to the lysate and shear samples in a bead mill for 1–3 min at maximum frequency. Avoid foaming by leaving only a small air bubble in the tube.

   ▲ **Note:** Make sure that no fuzzy material or clumps remain in the lysate.

5. Place a Multi-96 Filter or up to six Multi-8 Filter in a Multi-8 Filter Frame on top of the Multi-8/96 Columns. Split the lysate by applying each half on top of one filter of a Multi-8/96 Filter. Let the lysate pass through. Magnetically labeled mRNA is retained in the Multi-8/96 Columns.

   ▲ **Note:** Filtering the lysate removes cell debris and avoids the risk of clogging the column.

6. Apply 200 µL of Lysis/Binding Buffer onto the Multi-8/96 Filter. Remove drops from the tips of the Multi-8/96 Filter by moving the Multi-8/96 Filter against the inner wall of the Multi-8/96 Columns and discard Multi-8/96 Filter.

7. Proceed with section 2.3, Wash in the MultiMACS mRNA Isolation user manual.

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