Miltenyi Biotec

PrepProtect

10 mL	130-092-643
100 mL	130-092-642

Contents

1. Description

This product is for research use only.

Components 10 mL PrepProtect (# 130-092-643)
or
100 mL PrepProtect (# 130-092-642).

Storage Store bottle at room temperature. Close bottle immediately after withdrawal of a buffer aliquot and keep it tightly closed when not in use. The expiration date is indicated on the vial label.

1.1 Principle of RNA stabilization

High-quality RNA is a prerequisite for many gene expression profiling and cDNA cloning techniques like reverse transcription, real-time PCR, microarrays, Northern blot analysis, nuclease protection assays, and cDNA library construction. However, with its molecular characteristics, RNA is prone to chemical and enzymatical degradation. Degradation by ubiquitous RNases may, for example, occur during sample collection, storage, or RNA preparation. Additionally, when thawing frozen tissue for weighing, cutting, and homogenization the amount of intact RNA can be significantly reduced.

PrepProtect is a non-toxic reagent for stabilization of RNA in biological samples like cells and tissues. Addition of PrepProtect prevents RNA degradation, thereby protecting the RNA integrity and preserving the expression profile without cell lysis. Stabilized samples can then be used for RNA preparation, e.g. using µMACS™ or MultiMACS™ mRNA Isolation Kits.

1.2 Specifications and compatibility

The reagent can be used for two purposes:

A. Stabilization of samples prior to freezing

PrepProtect stabilizes freshly dissected tissue or harvested cells (fig. 1).

This allows samples to be stored or shipped at temperatures above –20 °C.

After stabilization of freshly dissected tissues or cultured cells in PrepProtect, samples can be stored in PrepProtect up to one day at 37 °C, up to one week at 25 °C, up to one month at 4 °C or indefinitely at –20 °C or below.

Stabilization for RNA isolation

The maximum RNA stabilization time depends on the RNase content of cells or tissue and might vary. As stabilization of tissue is limited by the penetration of the buffer, cut the tissue into slices with a maximum thickness of 5 mm.

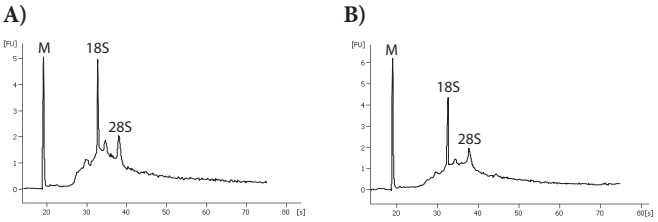

Figure 1: An amount of 10^6 Jurkat cells was frozen (A) or incubated (B) for one day at 37 °C in PrepProtect and mRNA was isolated with µMACS mRNA Isolation Kit. One µL of each sample was loaded on Agilent Bioanalyzer 2100.

Stabilization for protein isolation

PrepProtect preserves proteins in cell or tissue samples. As proteins are denatured by PrepProtect, PrepProtect-stored samples are suitable for applications that use denatured proteins like SDS gel electrophoresis.

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B. Stabilization of quick-frozen tissue

PrepProtect can be used to stabilize already frozen samples at –20 °C in order to store or thaw them without degradation. Samples become sliceable and can be handled or stored up to one hour at room temperature or overnight at 4 °C without degradation (fig. 2).

Therefore, after stabilization in PrepProtect frozen samples can be cut, weighed, and lysed by mechanical tools without prior crushing with mortar/pestle.

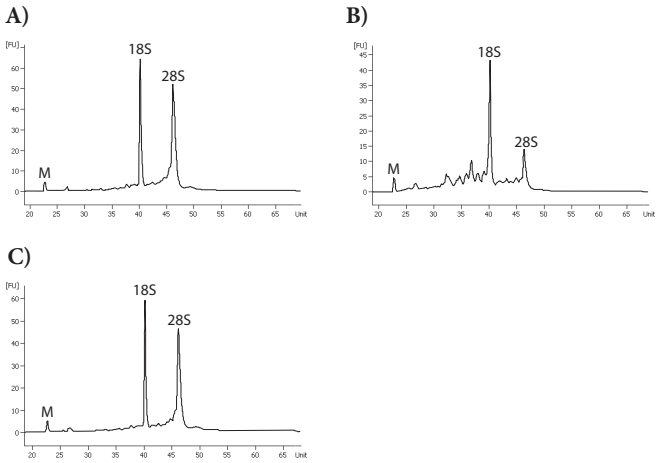

Figure 2: Quick-frozen liver sample was handled according to the table below and homogenized with a rotor-stator homogenizer. Total RNA was isolated with a silica-based spin kit and analyzed on an Agilent Bioanalyzer 2100.

Method of handling	RIN*	28S/18S
a) Crushed with mortar/pestle	8,8	1,4
b) Thawed 5 min at room temperature	6,1	0,4
c) Stabilized in PrepProtect and thawed 5 min at room temperature	8,8	1,3

*RIN: RNA Integrity Number calculated by Agilent Bioanalyzer Software.

1.3 Related products

A complete list of MACSmolecular products and protocols is available at www.miltenyibiotec.com.

µMACS products	Quantity	Column type	Order no.
µMACS mRNA Isolation Kits			
Small Scale	10 isolations	µ	130-090-276
Small Scale	20 isolations	µ	130-075-201
Large Scale	4 isolations	M	130-090-277
Large Scale	8 isolations	M	130-075-201
MultiMACS mRNA Isolation Kits			
(12×8)	12×8 isolations	Multi-8	130-092-520
(4×96)	4×96 isolations	Multi-96	130-092-519

2. Protocol for stabilization of samples prior to freezing

2.1. Stabilization of samples prior to freezing

Adjust PrepProtect to room temperature (20–22 °C).

Freshly dissected tissue

1. After dissection, estimate the volume of the sample. If necessary cut the tissue into slices with a maximum thickness of 5 mm.
2. Immediately submerge tissue completely in at least 5–10 volumes of PrepProtect and invert the tube several times.
   ▲ **Note:** When placing the tissue into the tube avoid sticking of tissue to the lid, side, or bottom. Optimally, the tissue piece is free-floating in PrepProtect.
3. Incubate tissue piece overnight at 4–22 °C.

Isolated or cultured cells

1. For adherent cells only: Remove the entire cell culture medium, wash cells once with cold PBS (4 °C), then detach cells, e.g. by trypsinization.
2. Spin down cells in a tube or plate according to your laboratory protocol, e.g. 5 min 300×g, and remove cell culture medium completely.
3. For detached cells by trypsinization: Wash cell pellet once with cold PBS (4 °C).
4. Resuspend cells in a small volume, e.g. for 10^6 cells, in 50 µL of PBS on ice.
5. Take tube out of the ice, add at least 5–10 volumes (minimum volume: 200 µL) of PrepProtect to the cell suspension and mix by inverting the tube five times. Spin shortly to collect the sample at the bottom.
   ▲ **Note:** If stabilization is done in microtiter plates, shake the plate shortly to re-suspend the cell pellet, add 200 µL PrepProtect, and shake again the plate shortly.

White blood cells (from whole blood)

Separate white blood cells out of whole blood with the autoMACS® Separator or lyse erythrocytes (see data sheets and special protocols at www.miltenyibiotec.com).

▲ All steps of red blood cell lysis have to be done at 4 °C.

Proceed with stabilization for **isolated or cultured cells.**

2.2 Storage or shipment

Store sample up to one day at 37 °C, up to one week at 25 °C, up to one month at 4 °C, or indefinitely at –20 °C or below.
For transport, ensure that the sample remains submerged and keep the tube upright. We recommend filling the tube completely with PrepProtect to ensure that the sample is always submerged in stabilization buffer. For sample shipment longer than two days, packing sample with cool packs (≤ 2–8 °C) into a styrofoam box is recommended.
For archival storage, incubate the sample (e.g. tissue after dissection, cells after isolation/detachment) in PrepProtect at 4 °C overnight, then store at –20 °C or –80 °C. Freshly stabilized samples kept at –20 °C or –80 °C can be thawed at room temperature and frozen again up to 20 times.

▲ **Note:** At –80 °C PrepProtect is frozen. This does not influence the stabilization.

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2.3 Preparation for RNA isolation

Stabilized tissue

1. Take samples out of PrepProtect with RNase-free forceps. Cut out an appropriate piece, remove excess liquid and weigh the sample. Store the remaining tissue in PrepProtect.

   ▲ **Note:** Freshly dissected tissue becomes harder in PrepProtect. Use half of the maximum sample amount specified in the RNA isolation protocol.

2. Lyse tissue with

   A) Mortar/pestle and rotor-stator homogenizer

   Crush sample with mortar/pestle before homogenization with a rotor-stator homogenizer.

   ▲ **Note:** For many samples addition of liquid nitrogen is not necessary! Although samples do not become fully pulverized, this does not interfere with the RNA isolation. Transfer crushed sample to a tube, add lysis buffer (refer to your RNA isolation protocol), and homogenize immediately with a rotor-stator homogenizer.

   B) Bead mill

   Put samples in an appropriate tube (2 mL) or 8-well strip (1.2 mL, for parallel processing). Fill cavity with two stainless steel beads, 5 mm diameter, and add lysis buffer (refer to your RNA isolation protocol). Close cavity and lyse samples immediately by starting the bead mill at highest setting for 2–5 min. Open cavity carefully or centrifuge before opening.

3. For µMACS/MultiMACS mRNA Isolation Kits: Incubate lysate 5 min at 70 °C. Let samples cool down for 10 min at room temperature and proceed to filtration step (LysateClear Columns or Multi-8/96 Filter).

Stabilized cells

1. Let sample adjust to room temperature. Spin down cells for 3 min at 2000×g.

   ▲ **Note:** If cells cannot be pelleted under these conditions, centrifugation forces up to 5000×g can be applied.

2. Aspirate supernatant completely without touching the cell pellet.

   ▲ **Note:** We recommend to spin again shortly to remove remaining liquid, or to aspirate the supernatant by a constant vacuum flow, e.g. with a pasteur pipette.

3. Add lysis buffer (refer to your RNA isolation protocol) and lyse with intermitted vortexing for at least one minute.

4. Recommended steps in addition to the standard purification protocol:

   For µMACS/MultiMACS mRNA Isolation Kits: Incubate lysate 5 min at 70 °C. Let samples cool down for 10 min at room temperature and proceed to filtration step (LysateClear Column or Multi-8/96 Filter).

   For total RNA kits with silica-based spin columns: A DNase I incubation step on the column is recommended.

3. Stabilization of quick-frozen tissue

3.1 Stabilization of quick-frozen tissue

The frozen sample should not thaw unless stabilized in PrepProtect.

1. Estimate the volume of the frozen sample stored at –80 °C and prechill an appropriate amount of PrepProtect at –20 °C.

2. Submerge the frozen tissue sample quickly into at least 10 volumes of prechilled PrepProtect.

   ▲ **Note:** We recommend to fill the tube almost completely with reagent to ensure that the sample is always submerged in stabilization buffer.

   ▲ **Note:** Tissue can be placed directly from liquid nitrogen into prechilled PrepProtect.

3. Incubate sample in PrepProtect at –20 °C (minimum 24 h per 0.5 mm diameter tissue).

3.2 Preparation for RNA isolation

1. Take samples out of PrepProtect with sterile forceps. Cut out an appropriate piece, remove excess liquid, and weigh the sample. Store the remaining tissue in PrepProtect at –20 °C.

2. Add lysis buffer (refer to your RNA isolation protocol), and homogenize immediately with a rotor-stator homogenizer.

   ▲ **Note:** As tissue samples incubated with PrepProtect generally return to their original consistency after thawing, pulverization with mortar/pestle is no longer required.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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