


mRNA Isolation Kit for Blood/Bone Marrow

for isolating mRNA from blood or bone marrow lysates

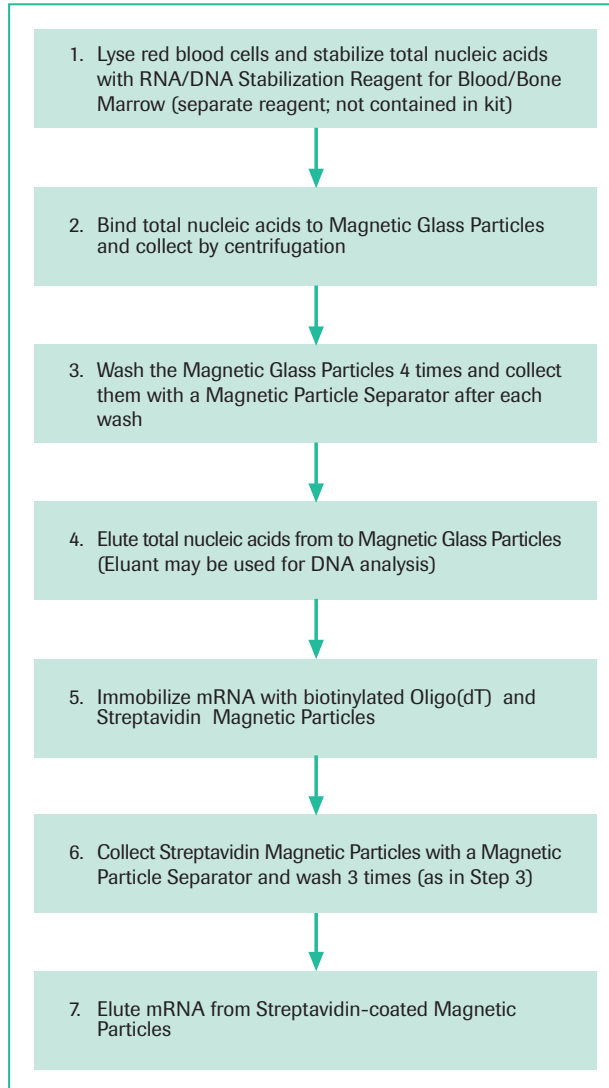
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Principle	<p>The purification of mRNA requires two steps:</p> <ol style="list-style-type: none"> 1. Cells from fresh blood or bone marrow are lysed with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. 2. After lysis, total nucleic acids released from blood or bone marrow are bound to Magnetic Glass Particles, washed free of cellular contaminants, then released from the Magnetic Glass Particles. Total nucleic acids are incubated with biotin-labeled oligo(dT) and Streptavidin-coated Magnetic Particles. The mRNA is bound to the Oligo(dT)-Streptavidin-coated Magnetic Particle complex, while other nucleic acids (DNA, rRNA, tRNA) are washed away. Purified mRNA is then released from the Streptavidin-coated Magnetic Particles. <p>The RNA/DNA Stabilization Reagent for Blood/Bone Marrow, described on the previous page, is used only for Step 1 of the procedure. The mRNA Isolation Kit for Blood/Bone Marrow, described on this page, is used only for Step 2 of the procedure.</p>
Starting material	<ul style="list-style-type: none"> ● Lysates containing nucleic acids from 1.5 – 5.0 ml human blood or bone marrow aspirates (research samples), obtained and stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow
Application	<ul style="list-style-type: none"> ● Isolation of highly purified, human mRNA, which is suitable for RT-PCR, cDNA synthesis, nuclease protection assays, Northern blotting, or Northern ELISA
Time required	<ul style="list-style-type: none"> ● Total time: 1.5 h (for isolation of mRNA from stabilized lysate) ● Hands-on time: approx. 1 h
Results	<ul style="list-style-type: none"> ● Typical mRNA yield: approx. 100 ng/ml of human blood ●  <i>Since white blood cell count can differ significantly between donors, mRNA yield can range from 50 to 200 ng/ml blood.</i> ● Variance in yield (aliquots from one donor): ≤10% ● Sensitivity: In a model system, it was possible to detect melanoma mRNA (by RT-PCR) in a kit-prepared sample containing mRNA from 5 ml of fresh blood that had been spiked with mRNA from 5 melanoma cells (Mel-Ju).
Benefits	<ul style="list-style-type: none"> ● Isolates nucleic acids without prior cell separation, ensuring representation of all cells in final mRNA isolate ● Increases the efficiency of RT-PCR screening by removing PCR inhibitors (rRNA, tRNA, and hemoglobin) from the mRNA template ● Allows detection of very rare mRNA species ● Produces high yields of purified, intact mRNA, suitable for multiple research applications ● Increases the reliability and reproducibility of mRNA isolation

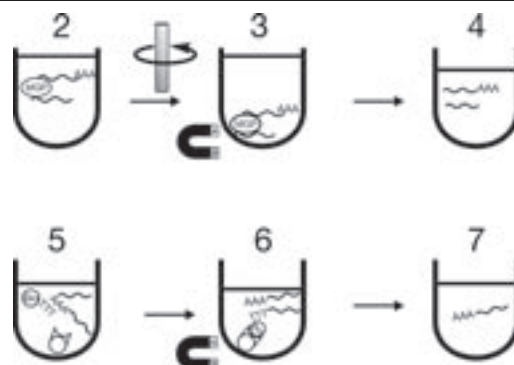
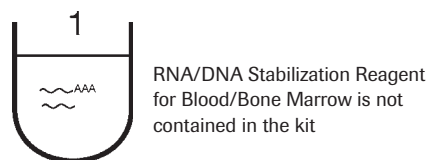
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How to use the reagent and the kit

I. Flow diagram



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II. Kit contents

- Magnetic Glass Particles (5 x 50 tablets)
- Magnetic Glass Particles Wash Buffer, 5x concentrated (2 x 20 ml)
 - ! Add 160 ml absolute ethanol to concentrated buffer to form the working Magnetic Glass Particle Wash Buffer used in Protocol IVb.
- Magnetic Glass Particles Elution Buffer (33 ml)
- Hybridization Buffer (17 ml)
- Oligo(dT)₂₀ Probe, biotin-labeled (330 µl)
- Streptavidin-coated Magnetic Particles (3.3 ml, suspension)
- Streptavidin Magnetic Particles Wash Buffer (110 ml)
- H₂O, double-distilled (18 ml)

III. Additional materials needed

- RNA/DNA Stabilization Reagent for Blood/Bone Marrow, with guanidine isothiocyanate and Triton X-100 (500 ml)
 - ! The reagent crystallizes at temperatures below 20°C. Check solution for the absence of crystals before use, and warm to 37°C if it is not fully dissolved. Thoroughly mix before use.
- Absolute ethanol
- Dry ice (optional, for short-term storage of sample lysates)
- Graduated cylinder or tube (for measuring blood/bone marrow volume)
- 50 ml centrifuge tubes, screw-cap, sterile
- Centrifuge for 50 ml tubes, capable of an 1100 x g centrifugal force
- 2 ml microcentrifuge tubes
- Tabletop microcentrifuge
- Magnetic Particle Separator
- Shaker for 2 ml tubes, for incubation at 37°C and 70°C
- Vortex mixer
- Roller incubator, for incubation at 20° to 25°C

IV. Protocols for preparing mRNA from 5 ml of human whole blood or bone marrow research samples



When handling blood, bone marrow, and blood/bone marrow lysates, take the precautions you usually take when handling potentially hazardous material. Dispose of all supernatants properly.

IVa. Lysis of cells from 5 ml of sample and stabilization of total nucleic acid

Sample preparation: Draw sample (blood or bone marrow) into a tube containing an anticoagulant (EDTA, citrate, or heparin). Label the tube with all relevant information (identification, kind of sample, total volume, date and time of draw, delay between draw and processing).

- 1 In a screw-cap container (bottle or tube) that will hold at least 55 ml:
 - ▶ Pour 50 ml prewarmed and fully dissolved RNA/DNA Stabilization Reagent for Blood/Bone Marrow.
 - ▶ Add 5 ml of sample.
 - ▶ Mix contents of container vigorously, for instance by vortexing.
- 2 Do one of the following:
 - ▶ If you are going to isolate mRNA immediately, go to Procedure IVb.
 - ▶ If you are going to store or transport the sample lysate to a central laboratory before isolating mRNA, go to Step 3.
- 3 Between now and the start of Procedure IVb, do one of the following:
 - ▶ Store lysate as follows:
 - ▶ Up to 1 year at -15 to -25°C
 - ▶ ≤1 day at 2 to 8°C.
 - ▶ ≤6 h at 20° to 25°C.
 - ▶ Place the lysate on dry ice for transport to another facility.

IVb. Isolation of mRNA from 55 ml of lysate (equivalent to 5 ml of sample)



For preparation of mRNA from small samples (1.5 – 3 ml blood or bone marrow), see the package insert supplied with the mRNA Isolation Kit for Blood/Bone Marrow.


Sample preparation: All lysates should be at 20 to 25°C. If the sample lysates have been frozen prior to this procedure, thaw them carefully and prewarm to 20 to 25°C. Thoroughly mix (e.g., by vortexing) to ensure crystallized material is fully dissolved.

- 1 Resuspend 8 Magnetic Glass Particles tablets in 480 µl double dist. H₂O.
- 2 In a sterile, screw-cap 50 ml centrifuge tube:
 - ▶ Add entire sample lysate from Procedure IVa.
 - ▶ Add Magnetic Glass Particles suspension from Step 1 and cap the tube.
 - ▶ Vortex for 10 s.
 - ▶ Incubate for 30 min at 20 to 25°C on a roller incubator.



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- 3 After the incubation:
- ▶ Centrifuge the tube for 2 min at 1100 x *g* and 15 to 25°C.
 - ▶ Discard the supernatant.
 - ▶ Invert the tube and place on filter paper for 30 s.
-  *Discard the supernatant properly, as you would any potentially hazardous material.*
- 4 Transfer the Magnetic Glass Particles to a 2 ml microcentrifuge tube, then wash them 4 times. For each wash:
- ▶ Resuspend Magnetic Glass Particles in 1 ml working Magnetic Glass Particle Wash Buffer with a pipette.
 - ▶ Collect the Magnetic Glass Particles on the side of the tube with a Magnetic Particle Separator.
 - ▶ Remove and discard all the supernatant.
 - ▶ Wait a few seconds, then remove (and discard) any residual supernatant.
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- 5 To elute the total nucleic acids from the Magnetic Glass Particles:
- ▶ Resuspend Magnetic Glass Particles in 1 ml Magnetic Glass Particle Elution Buffer.
 - ▶ Incubate suspension for 5 min on a 1400 rpm shaker at 70°C.
 - ▶ Collect the Magnetic Glass Particles on the side of the tube with a Magnetic Particle Separator.
 - ▶ Immediately transfer the supernatant to a fresh 2 ml tube.
 - ▶ Again use a Magnetic Particle Separator to remove any residual Magnetic Glass Particles from the supernatant.
 - ▶ Immediately transfer the supernatant to a fresh 2 ml tube. This supernatant contains the total nucleic acids from the sample.
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- 6 To isolate mRNA from the total nucleic acids:
- ▶ Prepare 100 µl Streptavidin Magnetic Particles: Transfer 100 µl SMP suspension to a fresh 2 ml tube. With a Magnetic Particle Separator, collect the Streptavidin Magnetic Particles from the suspension on the side of the tube. Withdraw and discard the supernatant (storage solution) from the tube.
 - ▶ Prepare 0.5 ml Hybridization Reagent: Add 10 µl biotin-labeled Oligo(dT)₂₀ Probe to 0.5 ml Hybridization Buffer and mix thoroughly.
 - ▶ Add 0.5 ml Hybridization Reagent to the total nucleic acids supernatant (from Step 5) and mix. Incubate for 2 min at 37°C.
 - ▶ Transfer the Hybridization Reagent-nucleic acid supernatant mixture to the tube containing 100 µl Streptavidin Magnetic Particles and resuspend the Streptavidin Magnetic Particles with a pipette.
 - ▶ Let the tube stand for 5 min at 37°C.
 - ▶ Collect Streptavidin Magnetic Particles on the side of the tube for 3 min with a Magnetic Particle Separator.
 - ▶ Discard the supernatant.
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- 7 Wash the Streptavidin Magnetic Particles 3 times. For each wash:
- ▶ Resuspend Streptavidin Magnetic Particles in 0.3 ml Streptavidin Magnetic Particle Wash Buffer with a pipette.
 - ▶ Collect the Streptavidin Magnetic Particles on the side of the tube with a Magnetic Particle Separator.
 - ▶ Remove and discard all the supernatant.
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- 8** To elute the mRNA from the Streptavidin Magnetic Particles:
- ▶ Resuspend Streptavidin Magnetic Particles in 20 µl double dist. H₂O with a pipette.
 - ▶ Let the tube stand for 2 min at 70°C.
 - ▶ Collect the Streptavidin Magnetic Particles on the side of the tube with a Magnetic Particle Separator.
 - ▶ Immediately transfer supernatant to a fresh 2 ml tube.
 - ▶ Again use a Magnetic Particle Separator to remove any residual Streptavidin Magnetic Particles from the supernatant.
 - ▶ Immediately transfer the supernatant to a fresh 2 ml tube.
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- 9** The supernatant (from Step 8) contains the mRNA from the sample.
You may:
- ▶ EITHER use the isolated mRNA directly in RT-PCR or other applications.
 - ▶ OR store the isolated mRNA at -15 to -25°C or -80°C.
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V. Troubleshooting the Stabilization and the mRNA Isolation protocol

If you get...	Then, the cause may be...	And you should...
Low yield of mRNA (<50 ng/ml blood)	RNase contamination in reagents or equipment	▶ See the Appendix, page 219 of this manual, for general guidelines on handling RNA.
	Improper storage of lysate before mRNA isolation	For optimal mRNA stability, store lysates: <ul style="list-style-type: none"> ▶ ≤1 year at -15 to -25°C ▶ ≤1 day at 2 - 8°C ▶ ≤6 h at 20 - 25°C
	Improper storage of blood before stabilization	Add RNA/DNA Stabilization Reagent for Blood/Bone Marrow to blood within a few hours after drawing.
Fragmented mRNA	RNase contamination in reagents or equipment	See the Appendix, page 219 of this manual, for general guidelines on handling RNA.
	Improper storage of lysate before mRNA isolation	For optimal mRNA stability, store lysates: <ul style="list-style-type: none"> ▶ ≤1 year at -15 to -25°C ▶ ≤1 day at 2 - 8°C ▶ ≤6 h at 20 - 25°C

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Typical results with the kit

Experiment 1

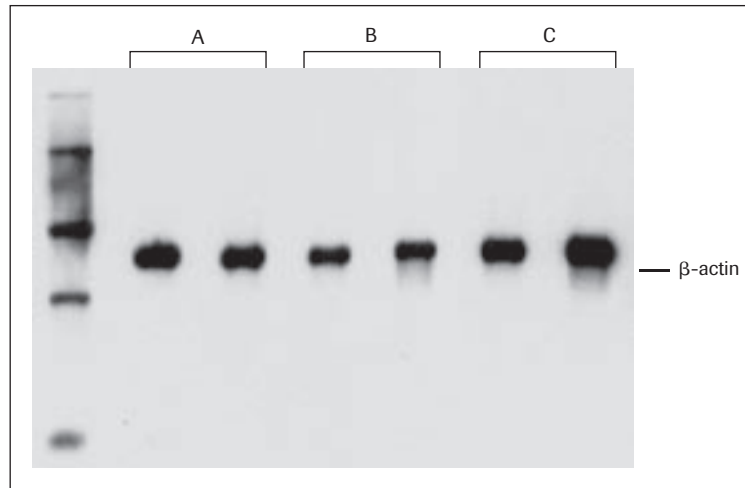


Figure 51: Stability of mRNA after lysis in RNA/DNA Stabilization Reagent for Blood/Bone Marrow. A total of 6 ml normal human heparinized blood research sample was lysed and stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. The lysate was divided into three aliquots.

Lanes A: One aliquot was stored at 2 to 8°C

Lanes B: One aliquot was stored at 15 to 25°C

Lanes C: One aliquot was stored at -15 to -25°C

After 4 days, duplicate samples were taken from each aliquot and mRNA was isolated from each, according to Protocol IVb. The isolated mRNA was separated electrophoretically, transferred to a membrane by Northern blotting, and analyzed with a DIG-labeled antisense β -actin RNA probe.

Result: The same band was clearly visible in each sample. However, the sample stored at -15 to -25°C (lanes C) contained more of the band than the sample stored at 2 to 8°C (lanes A), which in turn contained more than the sample stored at 15 to 25°C (lanes B).

Experiment 2

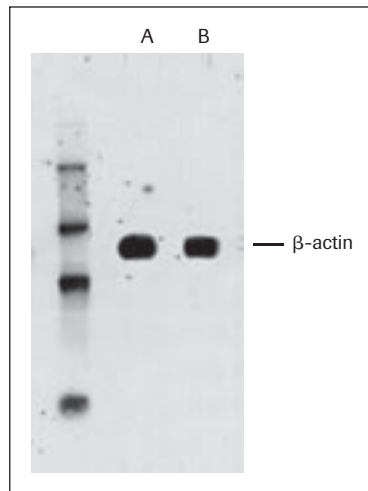


Figure 52: Isolation of mRNA from bone marrow and blood (research samples). Normal human heparinized bone marrow and blood were lysed with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. mRNA was isolated from each sample with the mRNA Isolation Kit for Blood/Bone Marrow. The mRNA from 0.8 ml bone marrow (**Lane A**) and from 1.0 ml of blood (**Lane B**) was separated electrophoretically, transferred to a membrane by Northern blotting, and analyzed with DIG-labeled antisense β -actin RNA probe.

Result: A single, sharp β -actin band was visible in each sample, which indicates the isolated mRNA was undegraded.

Reference

- Cools, J. et al. (2002) *Blood*, **99**, 1776 – 1784
- Gault, J. et al. (2005) *Stroke*, **36**, 872 – 874
- Hansen, I. A. et al. (2004) *Endocrinology*, **145**, 1898 – 1905
- Kakinuma, S. et al. (2003) *Stem Cells*, **21**, 217 – 227
- Lambooy, L. H. J. et al. (2003) *Clin. Cancer Res.*, **9**, 812
- Leying, H. et al. (1998) *Recent progress in molecular biology*, adapted from a poster at the Annual Meeting of the American Ass. For Cancer Research, New Orleans
- Noppen, C. et al. *Biochemica* (1997) **4**, 11-13, *Biochemica Information* (1998), **102**, 25 – 26
- Reinhold, U. et al. (2001) *J. Clin. Oncol.*, **19**, 1723 – 1727
- Seifert, T. et al. (2002) *Multiple Sclerosis*, **8**, 447 – 451
- Suárez, A. et al. (2005) *Ann Rheum Dis*, **64**, 1605 – 1610