For life science research only.
Not for use in diagnostic procedures.

nt Protocol nt Changes to

mRNA Isolation Kit for Blood/Bone Marrow

Version 6.0

Content version: October 2010

For isolation of mRNA from blood or bone marrow lysates prepared by using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow

Cat. No. 11 934 333 001

100 (50, 30) isolations
From 1.5 ml (3 ml; 5 ml)
of sample material

Store the kit at +2 to +8°C

www.roche-applied-science.com

Table of contents

CAUTION

The following reagent, which is recommended in this instruction manual, is toxic or corrosive and should be handled with care:
RNA/DNA Stabilization Reagent for Blood/Bone Marrow (contains guanidinium isothiocyanate and Triton X-100) (Cat. No. 11 934 317 001)
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.

Product Description

- The mRNA Isolation Kit for Blood/Bone Marrow relies on magnetic glass particle technology. It uses peripheral blood or bone marrow samples stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow* and follows a two-step procedure: (i) total nucleic acid fraction is prepared by taking advantage of their ability to non-specifically adsorb to glass (silica) surfaces, and (ii) mRNA fraction is purified by virtue of hybridization to biotin-labeled oligo (dT) and capturing by streptavidin-coated magnetic particles, followed by magnetic separation.
- Total nucleic acids are prepared intermediately and can be used for DNA analyses like PCR.

Number of Tests

- 100 isolations from 1.5 ml of sample material (16.5 ml of stabilized lysate)
- 50 isolations from 3 ml of sample material (33 ml of stabilized lysate)
- 30 isolations from 5 ml of sample material (55 ml of stabilized lysate)

Application

mRNA isolation is a prerequisite for a variety of different applications like

- Reverse Transcription Polymerase Chain Reaction (RT-PCR),
- Northern blotting,
- Nothern ELISA,
- ribonuclease protection assay, and
- preparation of cDNA libraries.

In addition to these general applications, the mRNA Isolation Kit for Blood/Bone Marrow is particularly useful for the

- detection of rare cells like disseminated tumor cells by RT-PCR, because of the following features:

a. Omission of cell separation steps that could result in a loss of rare target cells,

b. Option to isolate nucleic acids from large sample volumes,

c. Option to use the mRNA isolated from a large sample volume for one single RT-PCR reaction,

d. Isolation of mRNA instead of total RNA, which inhibits the RT-PCR reaction if microgram amounts are used,

e. Small elution volume,

f. Independence from added anti-coagulants (heparin, EDTA, citrate).

Stability

The kit is stable at +2 to +8°C until the expiration date printed on the label.
For stability of solutions, see section 5.1.

continued on next page

Quality Control

The kit is function-tested for the detection of melanoma cells in normal human blood.

Model system: detection of tyrosinase mRNA by RT-PCR after addition of MelJu cell mRNA to fresh normal human blood stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. The mRNA is purified from the stabilized lysate with the mRNA Isolation Kit for Blood/Bone Marrow, as described below (see: section 6.1).

Recovery of MelJu mRNA is shown by detection of tyrosinase mRNA by RT-PCR.

Advantages

- **Omission of cell separation prior to mRNA isolation:** mRNA and total nucleic acids can be isolated from blood or bone marrow lysates.
- **Easy stabilization and stable storage after sample collection:** blood or bone marrow is stabilized by the addition of RNA/DNA Stabilization Reagent for Blood/Bone Marrow* and can be stored for 12 months at −15 to −25°C or for one day at +2 to +8°C.
- **Flexible sample volume:** the kit is suitable for sample volumes of 1.5 ml (or less) and up to 5 ml.
- **Small elution volume:** mRNA isolated from 5 ml of blood can be eluted in 20 µl of redistilled water.
- **mRNA isolated from a large sample volume can be used for one single RT-PCR reaction without any signs of inhibition:** mRNA from 4 ml of sample material can be used entirely for one RT-PCR reaction regardless of the added anti-coagulant (heparin, EDTA, citrate).
- **High quality mRNA:** the procedure guarantees isolation of intact mRNA.
- **High yield:** dependent on the sample donor, between 50–200 ng of mRNA can be isolated from 1 ml of human blood.
- **Excellent reproducibility:** Variance in yield ≤10%.

RNA Instability

- **Ensure, that the material getting in contact with RNA is free of contaminating RNases**. The extreme instability of RNA is mainly due to the ubiquitous presence of enzymes (RNases) which degrade RNA and can recover activity even after many forms of treatment such as boiling (1). Protocols for decontamination of equipment are described in reference 1.
- **Disrupt cells and stabilize RNA (by inactivation of RNases) as soon as possible after sample collection**. To obtain good preparations of eukaryotic mRNA, it is necessary to minimize the activity of RNases liberated during cell lysis by using methods that disrupt cells and inactivate RNases simultaneously (1).
- **Do not store blood or bone marrow samples for more than a few hours, without stabilization**. Even in their natural environment within the cell, most mRNAs are extremely unstable. The storage of cells in an "artifical" environment results in qualitative and quantitative changes of the mRNA content of the cells.

RNA Stabilization

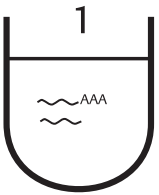
- An excellent way to protect against RNase-mediated degradation than to **disrupt cells in guanidinium lysis buffers** (2; 3).
- RNA lysis buffers that contain guanidinium thiocyanate or guanidinium-HCl consistently yield the highest quality sample (3).

Rare Cell Detection

RT-PCR allows the detection of disseminated tumor cells in blood or bone marrow with unprecedented sensitivity (4; 5). Nevertheless, some aspects particularly concerning sample stabilization and other steps preceeding the actual mRNA isolation procedure should be considered carefully:

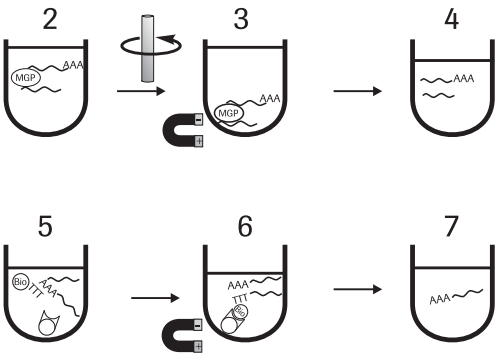
- **Omit cell separation steps** to proactively avoid loss of target cell, whenever possible. Particularily tumor cells and micrometastases are heterogeneous. There is no one tumor and the behaviour of tumor cells or micrometastasis during cell separation is clearly not predictable.
- **Use mRNA instead of total RNA**. For ultra-sensitive detection of rare cells by RT-PCR, the mRNA isolated from a large sample volume (*e.g.*, 1 ml) should be used for one single RT-PCR reaction. One ml of normal human blood contains 4–20 µg of total RNA which is far too much to use in one RT-PCR reaction. In the case of mRNA, the corresponding amount (100 – 400 ng) does not result in decreased sensitivity.
- **Use enough mRNA for detection in one RT-PCR reaction.** Assuming that one tumor cell containing 10 target transcripts has to be detected in the presence of 10^7 white blood cells (2 ml of blood), it is necessary to use at least the mRNA of 2 ml of blood in one single RT-PCR reaction.
- **Be sure that the RNA isolation method efficiently purifies nucleic acids away from inhibitors of the RT-PCR reaction**. Blood and bone marrow samples contain lots of potent inhibitors of the RT-PCR reaction, like hemoglobin and the anti-coagulant heparin. Especially, if the isolated RNA is not diluted for RT-PCR, the efficiency of the purification procedure is of extreme importance.

Stabilization of RNA/DNA from blood/bone marrow by adding the sample to stabilization reagent



RNA/DNA Stabilization Reagent for Blood/Bone Marrow is not contained in the kit

Isolation of mRNA from blood or bone marrow lysate



Principle

The isolation procedure is based on (magnetic) bead technology and can be devided into the following steps:

1. Lysis and stabilization of the sample with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow (not contained in the kit).
2. Magnetic glass particles (MGPs) are added to a blood or bone marrow lysate, and total nucleic acids (RNA, DNA) are bound onto the MGPs during incubation.
3. MGPs are separated by centrifugation or magnetic force and unbound material is removed by washing.
4. Nucleic acids are eluted from the MGPs. At this stage, part of the nucleic acids can be used for DNA analysis.
5. mRNA is captured from total nucleic acids by using biotin-labeled oligo(dT) and streptavidin-coated magnetic particles (SMPs).
6. SMPs are separated by magnetic force and unbound material is removed by washing.
7. mRNA is eluted after removal of other nucleic acids (DNA, rRNA, tRNA) by washing.

4. Kit characteristics

Sample Material	• Lysates of EDTA-, citrate-, or heparin-blood, which were prepared by using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow*. • Lysates of EDTA-, citrate-, or heparin-containing bone marrow, which were prepared by using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow*.
Yield	≥ 50 ng (50–200 ng) mRNA/ml of normal human blood (depending on the blood donor)
Sample Volume	≤ 1.5–5 ml of blood or bone marrow corresponding to ≤ 16.5–55 ml of lysate prepared by using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow*.
Variance in Yield	≤ 10% (evaluated from OD measurement after isolation of mRNA from sample aliquots of one donor)
Time Required	1.5 hours for 3 samples
Hands-on Time Required	≤ 1.0 hour for 3 samples

5. Kit Contents, Preparation of Working Solutions, Additionally Required Solutions, and Equipment

5.1 Kit Contents and Preparation of Working Solutions

Bottle/ Cap	Label	Content	Working solution	Preparation of working solution	Stability of working solution	For use in
1 white	MGP (Magnetic Glass Particles)	5 bottles each containing • 50 tablets; one tablet contains 7.5 mg MGP	Solution 1	Transfer the required number of tablets into a tube and add 60 µl of redistilled water (Solution 8) per tablet, suspend by pipetting immediately before use	unstable; do not store this supension for longer than 8 hours at +15 to +25°C	step 2
2 color-less	MGP washing buffer, 5×	2 bottles each containing • 20 ml	Solution 2	Add 80 ml of ethanol to each bottle	stable for 6 month at +15 to +25°C	step 5
3 red	MGP elution buffer	33 ml solution	Solution 3	ready-to-use	see: kit control date	step 6
4 white	Hybridization buffer	17 ml solution	Solution 4	ready-to-use	see: kit control date	Solution 9
5 violet	Oligo$(dT)_{20}$ probe, biotin-labeled	330 µl solution (10 pmol/µl)	Solution 5	ready-to-use	see: kit control date	Solution 9
6 orange	SMP (Streptavidin Magnetic Particles	3.3 ml suspen-sion (10 mg/ml)	Solution 6	Resuspend before use. Remove storage buffer as described (see procedure)	see: kit control date	step 7
7 green	SMP wash-ing buffer	110 ml solution	Solution 7	ready-to-use	see: kit control date	step 8
8 blue	Redistilled water	18 ml solution	Solution 8	ready-to-use	see: kit control date	step 9

5.2 Additionally Required Solutions and Equipment

Solutions

<table>
<tr><th>Solution</th><th>Preparation</th><th>Stability</th><th>For use in</th></tr>
<tr><td>Solution 9
Hybridization reagent</td><td>Add 50 vol. of Solution 4 to 1 vol. of Solution 5. Depending on the sample size, the following volumes are required per sample:
<table>
<tr><th>Sample size</th><th>Solution 4</th><th>Solution 5</th></tr>
<tr><td>5 ml</td><td>0.5 ml</td><td>10 µl</td></tr>
<tr><td>3 ml</td><td>0.3 ml</td><td>6 µl</td></tr>
<tr><td>1.5 ml</td><td>0.15 ml</td><td>3 µl</td></tr>
</table></td><td>stable for two weeks at +2 to +8°C, and 8 h at 30°C</td><td>step 7</td></tr>
</table>

- Ethanol (p. a., or analytical grade) for preparation of solution 2.

Equipment

- Centrifuge used for 50 ml tubes (*e.g.*, Falcon tubes) suitable for centrifugation at about 1,100 × *g*
- Bench-top centrifuge used for 2 ml tubes
- Magnetic separator suitable for 2 ml tubes
- Vortex mixer
- Roller incubator or other suitable instrument; incubation temperature: +15 to +25°C
- Shaker for tubes (*e.g.*, Eppendorf tubes) that allows incubation at 70°C

6. Procedure

Sample Material

- Blood lysates in RNA/DNA Stabilization Reagent for Blood/Bone Marrow*
- Bone marrow lysates in RNA/DNA Stabilization Reagent for Blood/Bone Marrow*

Note: Lysates are only suitable, if stored properly. Storage conditions:

- not longer than 12 months at −15 to −25°C,
- not longer than one day at +2 to +8°C
- not longer than 6 hours at +15 to +25°C.

Pre-Treatment of Samples

If lysates are deep-frozen, thaw samples carefully. Do not store the thawed samples at temperatures above 25°C.

Sample Volumes

In the following, the detailed procedures for mRNA isolation from 55 ml, 33 ml, and 16.5 ml of lysates corresponding to 5 ml, 3 ml, and 1.5 ml of original sample material are described. If sample volumes less than 1.5 ml are to be processed, the procedure described for 1.5 ml can be linearly downscaled.

Quantification of mRNA

Determine the amount of isolated mRNA spectrophotometrically.

Calculate the difference between $A_{260\ nm}$ and $A_{320\ nm}$ ($A_{260nm} - A_{320\ nm}$).

1 $A_{260nm} - A_{320\ nm}$ unit corresponds to a concentration of 40 μg/ml.

continued on next page

6.1 Isolation of mRNA from 55 ml of Lysate, Corresponding to 5 ml of Stabilized Sample (Blood, Bone Marrow)

Procedure

Step	Action
1	Prewarm lysate to +15 to +25°C. Thoroughly mix (*e.g.*, by vortexing) to ensure that crystallized material is fully dissolved.
2	• To prepare Solution 1, Pre-suspend 8 tablets (bottle 1) in 480 µl of redist. water (Solution 8) • Transfer the lysate into a 50 ml Falcon tube and add Solution 1. • Vortex for 10 s
3	Bind nucleic acids to MGPs by incubation • for 30 min • at +15 to +25°C • on a roller incubator
4	Separate MGPs by centrifugation • for 2 min • at +15 to +25°C • at 1,100 × *g*. Discard supernatant and place the inverted tube on a filter paper for 30 s
5	Wash MGPs four times by using a magnetic separator and a suitable tube (*e.g.*, 2 ml Eppendorf tube). Washing steps are carried out as follows: • suspend separated MGPs by pipetting thoroughly in 1 ml MGP washing buffer (Solution 2) • separate MGPs by using a magnetic separator • remove supernatant completely, wait several seconds and remove the remaining supernatant again. **Note:** Do not wash by vortexing.
6	Resuspend MGPs in 1 ml of MGP elution buffer (Solution 3). Elute nucleic acids by incubation • for 5 min • at 70°C • at 1,400 rpm in a suitable shaker (*e.g.*, Eppendorf shaker). Separate MGPs using a magnetic separator and transfer supernatant instantly into a fresh tube. Repeat separation with the supernatant to remove residual MGPs completely and transfer supernatant into a fresh tube. Part of the isolated nucleic acids can be used for DNA analysis.

continued on next page

6.1 Isolation of mRNA from 55 ml of Lysate, Corresponding to 5 ml of Stabilized Sample (Blood, Bone Marrow)

Procedure

Step	Action
7	Transfer 100 µl (1 mg) resuspended SMPs (Solution 6) into a fresh tube and separate SMPs from storage solution (3 min) using a magnetic separator. Discard supernatant. Add 0.5 ml of Hybridization reagent (Solution 9) to the eluate from step 6 and mix by pipetting Transfer the sample to the tube, containing the SMPs and mix by pipetting. Incubate • for 5 min at 37°C. Separate magnetic particles for at least 3 min by using a magnetic separator and discard supernatant.
8	Wash magnetic particles three times by using a magnetic separator. Washing steps are carried out as follows: • suspend separated magnetic particles by pipetting in 0.3 ml of SMP washing buffer (Solution 7) • separate magnetic particles by using a magnetic separator • remove supernatant completely
9	Resuspend magnetic particles in 20 µl of redistilled water (Solution 8). Elute mRNA by incubation • for 2 min • at 70°C Separate magnetic particles using a magnetic separator and transfer supernatant into a fresh tube. Repeat separation with the supernatant to remove residual magnetic particles completely and transfer supernatant into a fresh tube.
10	Further process isolated mRNA, *e.g.*, in RT-PCR, or store at −20 to −80°C.

6.2 Isolation of mRNA from 33 ml of Lysate Corresponding to 3 ml of Stabilized Sample (Blood, Bone Marrow)

Procedure

Step	Action
1	Prewarm lysate to +15 to +25°C. Thoroughly mix (*e.g.*, by vortexing) to ensure that crystallized material is fully dissolved.
2	• To prepare solution 1, pre-suspend 5 tablets (bottle 1) in 300 µl of redist. water (Solution 8) • Transfer the lysate into a 50 ml Falcon tube and add Solution 1. • Vortex for 10 s
3	Bind nucleic acids to MGPs by incubation • for 30 min • at +15 to +25°C • on a roller incubator
4	Separate MGPs by centrifugation • for 2 min • at +15 to +25°C • at 1,100 × *g*. Discard supernatant and place the inverted tube on a filter paper for 30 s
5	Wash MGPs three times by using a magnetic separator and a suitable tube (*e.g.*, 2 ml Eppendorf tube). Washing steps are carried out as follows: • suspend separated MGPs by pipetting thoroughly in 1 ml MGP washing buffer (Solution 2) • separate MGPs by using a magnetic separator • remove supernatant completely, wait several seconds and remove the remaining supernatant again. **Note:** Do not wash by vortexing.
6	Resuspend MGPs in 0,6 ml of MGP elution buffer (Solution 3). Elute nucleic acids by incubation • for 5 min • at 70°C • at 1400 rpm in a suitable shaker (*e.g.*, Eppendorf shaker). Separate MGPs using a magnetic separator and transfer supernatant instantly into a fresh tube. Repeat separation with the supernatant to remove residual MGPs completely and transfer supernatant into a fresh tube. Part of the isolated nucleic acids can be used for DNA analysis.

continued on next page

6.2 Isolation of mRNA from 33 ml of Lysate Corresponding to 3 ml of Stabilized Sample (Blood, Bone Marrow)

Procedure

Step	Action
7	Transfer 60 µl (0.6 mg) resuspended SMPs (Solution 6) into a fresh tube and separate SMPs from storage solution (3 min) using a magnetic separator. Discard supernatant. Add 0.3 ml of Hybridization reagent (Solution 9) to the eluate from step 6 and mix by pipetting Transfer the sample to the tube, containing the SMPs and mix by pipetting. Incubate • for 5 min at 37°C. Separate magnetic particles for at least 3 min by using a magnetic separator and discard supernatant.
8	Wash magnetic particles two times by using a magnetic separator. Washing steps are carried out as follows: • suspend separated magnetic particles by pipetting in 1 ml of SMP washing buffer (Solution 7) • separate magnetic particles by using a magnetic separator • remove supernatant completely
9	Resuspend magnetic particles in 12 µl of redistilled water (Solution 8). Elute mRNA by incubation • for 2 min • at 70°C Separate magnetic particles using a magnetic stand and transfer supernatant into a fresh tube. Repeat separation with the supernatant to remove residual magnetic particles completely and transfer supernatant into a fresh tube.
10	Further process isolated mRNA, *e.g.*, in RT-PCR, or store • at −20 to −80°C.

6.3 Isolation of mRNA from 16.5 ml of lysate corresponding to 1.5 ml of Stabilized Sample (Blood, Bone Marrow)

Procedure

Step	Action
1	Prewarm lysate to +15 to +25°C. Thoroughly mix (*e.g.*, by vortexing) to ensure that crystallized material is fully dissolved.
2	• To prepare solution 1 pre-suspend 2 tablets (bottle 1) in 120 µl of redist. water (Solution 1) • Add suspension to the lysate which has been transferred into a 50 ml Falcon tube. • Vortex for 10 s
3	Bind nucleic acids to MGPs by incubation • for 30 min • at +15 to +25°C • on a roller incubator
4	Separate MGPs by centrifugation • for 2 min • at +15 to +25°C • at 1,100 × *g*. Discard supernatant and place the inverted tube on a filter paper for 30 s
5	Wash MGPs three times by using a magnetic separator and a suitable tube (*e.g.*, 1.5 ml Eppendorf tube). Washing steps are carried out as follows: • suspend separated MGPs by pipetting thoroughly in 0.5 ml MGP washing buffer (Solution 2) • separate MGPs by using a magnetic separator • remove supernatant completely, wait some seconds and remove the remaining supernatant again. **Note:** Do not wash by vortexing
6	Resuspend MGPs in 0.3 ml of MGP elution buffer (Solution 3). Elute nucleic acids by incubation • for 5 min • at 70°C • at 1,400 rpm in a suitable shaker (*e.g.*, Eppendorf shaker). Separate MGPs using a magnetic separator and transfer supernatant instantly into a fresh tube. Repeat separation with the supernatant to remove residual MGPs completely and transfer supernatant into a fresh tube. Part of the isolated nucleic acids can be used for DNA analysis.

continued on next page

6.3 Isolation of mRNA from 16.5 ml of Lysate Corresponding to 1.5 ml of Stabilized Sample (Blood, Bone Marrow)

Procedure

Step	Action
7	Transfer 30 µl (0.3 mg) resuspended SMPs (Solution 6) into a fresh tube and separate SMPs from storage solution (3 min) using a magnetic separator. Discard supernatant. Add 0.15 ml of Hybridization reagent (Solution 9) to the eluate from step 6 and mix by pipetting Transfer the sample to the tube, containing the SMPs and mix by pipetting. Incubate • for 5 min at 37°C. Separate magnetic particles for at least 3 min by using a magnetic separator and discard supernatant.
8	Wash magnetic particles two times by using a magnetic separator. Washing steps are carried out as follows: • suspend separated magnetic particles by pipetting in 0.5 ml of SMP washing buffer (Solution 7) • separate magnetic particles by using a magnetic separator • remove supernatant completely
9	Resuspend magnetic particles in 12 µl of redistilled water (Solution 8). Elute mRNA by incubation • for 2 min • at 70°C Separate magnetic particles using a magnetic separator and transfer supernatant into a fresh tube. Repeat separation with the supernatant to remove residual magnetic particles completely and transfer supernatant into a fresh tube.
10	Further process isolated mRNA, *e.g.*, in RT-PCR, or store • at −20 to −80°C.

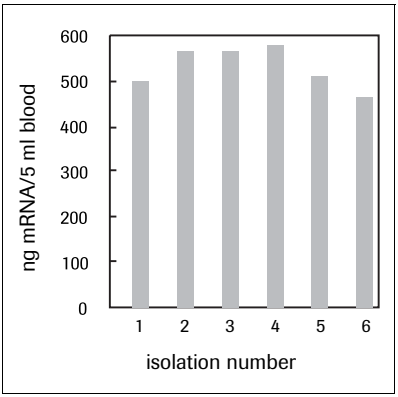
Introduction

The following figures show typical results regarding:

- mRNA yield and reproducibility
- Isolation of mRNA from 5 ml, 3 ml, and 1.5 ml of human blood
- Dependence of sensitivity on sample volume
- Isolation of mRNA from bone marrow
- Detection of melanoma cells in bone marrow (model system)
- Binding of total nucleic acids onto magnetic glass particles (MGPs)

mRNA Purification is Highly Reproducible

Fig. 1: 30 ml of normal human heparinized blood were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. mRNA was isolated from 6 aliquots corresponding to 5 ml of blood each using the mRNA Isolation Kit for Blood/Bone Marrow. The yield was calculated measuring at $A_{260\ nm}$ (1 $A_{260\ nm}$ unit corresponds to 40 µg mRNA/ml).



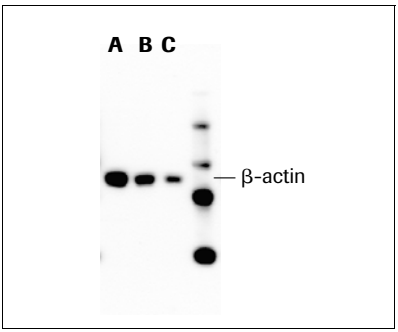
The Purified mRNA is Free from Signs for Degradation

Fig. 2: 5 ml, 3 ml, and 1.5 ml of normal human heparinized blood were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. The mRNA was isolated from each lysate using the mRNA Isolation Kit for Blood/Bone Marrow. 30% of each eluate were analyzed by Northern blotting using a DIG-labeled anti-sense RNA β-actin probe.

A: 5 ml blood

B: 3 ml blood

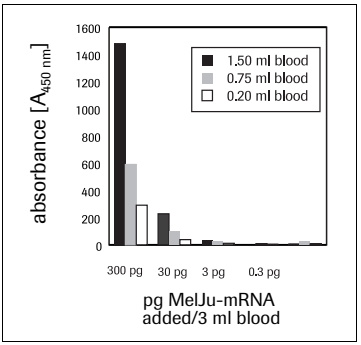
C: 1.5 ml blood



continued on next page

Sensitivity of Tumor Cell Detection Correlates with Sample Volume

Fig. 3: 12 ml of normal heparinized blood were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. To 4 aliquots of the lysate corresponding to 3 ml blood each, 300 pg, 30 pg, 3 pg, and 0.3 pg of melanoma cell line (MelJu) mRNA were added. The mRNA was isolated from each lysate using the mRNA Isolation Kit for Blood/Bone Marrow. Eluates corresponding to 1.5 ml, 0.75 ml, and 0.2 ml of blood were subjected to RT PCR using tyrosinase cDNA specific primers (HTYR1, HTYR2). The PCR products were detected by PCR ELISA.

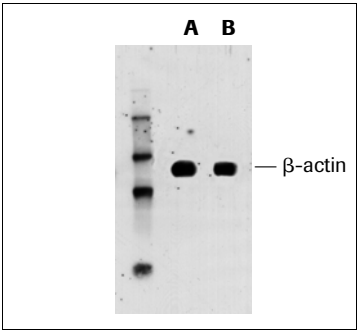


Comparable Results with Blood and Bone Marrow

Fig. 4: The human heparinized blood and bone marrow were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. The mRNA was isolated from each lysate using the mRNA Isolation Kit for Blood/Bone Marrow. mRNA corresponding to 0.8 ml of bone marrow and 1 ml of blood were analyzed by Northern blotting using a DIG-labeled anti-sense RNA β-actin probe.

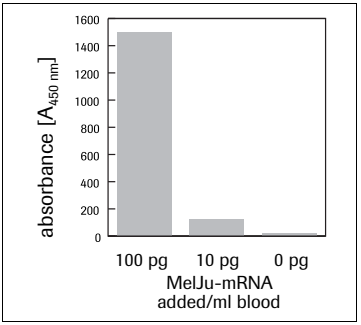
A: bone marrow

B: blood



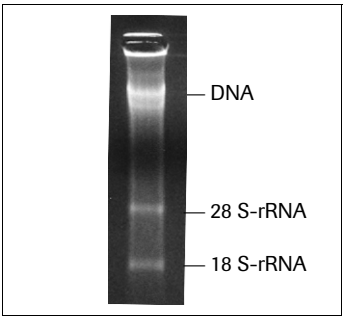
Bone marrow is suitable for tumor cell detection

Fig. 5: 3 ml of human heparinized bone marrow were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. To 3 aliquots of the lysate corresponding to 1 ml blood each, 100 pg, 10 pg, and 0 pg of melanoma cell line (MelJu) mRNA were added. The mRNA was isolated from each lysate using the mRNA Isolation Kit for Blood/Bone Marrow. The eluate was subjected to RT PCR using tyrosinase cDNA specific primers (HTYR1, HTYR2). The PCR products were detected by PCR ELISA.



Magnetic Glass Particles Bind Total Nucleic Acids

Fig. 6: Blood cells were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Total nucleic acids were bound and eluted from magnetic glass particles (MGPs) and analyzed by agarose gel electrophoresis.



8. Troubleshooting

Problem	Possible Cause	Recommendation
Low yield of mRNA (*e.g.*, <50 ng/ml of human blood)	• Lysate has not been stored properly before isolation of mRNA	Stability of lysates: • up to 12 months at −15 to −25°C, • up to one day at +2 to +8°C • up to 6 hours at +15 to +25°C. Store lysates accordingly.
	• Blood has not been stored properly before stabilization	Storage of whole blood results in continuous loss of mRNA. Avoid storage of unstabilized samples for more than a few hours.
Integrity of isolated mRNA is not appropriate	• Lysate has not been stored properly before isolation of mRNA	Stability of lysates: • up to 12 months at −15 to −25°C • up to one day at +2 to +8°C • up to 6 hours at +15 to +25°C. Store lysates accordingly

9. References

1 Sambrook J., Fritsch E. F., & Maniatis T. (1989) Molecular Cloning: *A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

2 Chirgwin J. M. et al. (1979) *Biochemistry* **18,** 5294

3 Farrell R. E. (1993) RNA Methodologies: A Laboratory Guide for Isolation and Characterization, Academic Press, San Diego.

4 Smith et al. (1991) *Lancet* **338,** 1227.

5 Moreno et al. (1992) *Cancer Res.* **52,** 6110.

10. Ordering Information

Kits

Product	Pack size	Cat. No.
mRNA Capture Kit	1 kit (192 reactions)	11 787 896 001
mRNA Isolation Kit	1 kit for the isolation of at least 70 μg poly(A^+) RNA	11 741 985 001
DNA Isolation Kit for Mammalian Blood	1 kit (25 purifications of 10 ml samples)	11 667 327 001

Single Reagents

Product	Pack size	Cat. No.
RNA/DNA Stabilization Reagent for Blood/Bone Marrow	500 ml for 50 ml sample material	11 934 317 001
Red Blood Cell Lysis Buffer	10 ml for 250 – 500 reactions	11 814 389 001
Streptavidin-coated PCR Tubes (Strips)	24 strips of 8 × 0.2 ml streptavidin-coated tubes and 24 strips of 8 caps	11 741 772 001
Tth DNA Polymerase	100 units	11 480 014 001
Titan One Tube RT-PCR System	100 reactions 25 reactions	11 855 476 001 11 888 382 001
Transcriptor Reverse Transcriptase	250 units (25 reactions) 500 units (50 reactions) 4 × 500 units (200 reactions	03 531 317 001 03 531 295 001 03 531 287 001

11. Changes to Previous Version

Discontinuation of Magnetic Particle Separator

Editorial changes

Contact and Support

If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the world-wide research community.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site at:**

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.
Country-specific contact information will be displayed.
On the Roche Applied Science home page select **Printed Materials** to find:

- in-depth Technical Manuals
- Lab FAQS: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

1010.**11924109001**©

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany