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

RNA/DNA Stabilization Reagent for Blood/Bone Marrow

Version: 07
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For simultaneous cell lysis and stabilization of nucleic acids in blood or bone marrow samples

Cat. No. 11 934 317 001	1 bottle 500 ml for up to 50 ml sample material

Store the reagent at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	RNA/DNA Stabilization Reagent for Blood/Bone Marrow	▪ Contains guanidinium thiocyanate, Triton X-100, and a reducing chemical. ▪ Ready-to-use solution.	1 bottle, 500 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the reagent is stable through the expiration date printed on the label.

Vial / bottle	Label	Storage
1	RNA/DNA Stabilization Reagent for Blood/Bone Marrow	Store at +15 to +25°C. ⚠ ***Reagent crystallizes at temperatures <+20°C. If precipitates are present, warm to +37°C until dissolved. Mix well before use.***

1.3. Additional Equipment and Reagent required

Standard Laboratory Equipment

- Equipment required for the measurement of reagent and blood volumes.
- Bottles or tubes, such as Falcon tubes suitable for mixing the RNA/DNA Stabilization Reagent for Blood/Bone Marrow and sample material.
- Dry ice, if sample lysates must be transported.

1.4. Application

The RNA/DNA Stabilization Reagent for Blood/Bone Marrow is used for cell lysis and simultaneous inactivation of enzymes, such as ribonucleases that otherwise degrade RNA.
Samples processed using the reagent are suitable for the isolation of mRNA or total nucleic acids by, for example, the mRNA Isolation Kit for Blood/Bone Marrow*.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The RNA/DNA Stabilization Reagent can be used with human peripheral blood and bone marrow samples.

Commonly used anti-coagulants such as heparin, EDTA, or citrate do not interfere with sample stabilization, the mRNA isolation procedure using the mRNA Isolation Kit for Blood/Bone Marrow, or RT-PCR after mRNA purification.

Sample Volume

The sample or lysate volume that is compatible with the mRNA Isolation Kit for Blood/Bone Marrow ranges from ≤1.5 to 5 ml of blood or bone marrow, corresponding to ≤16.5 to 55 ml of lysate.

Storage of Lysates

The RNA/DNA Stabilization Reagent provides easy stabilization and stable storage; blood or bone marrow is stabilized by simply mixing the reagent and sample.

Lysates are only suitable if stored properly:
- Maximum of 12 months at −15 to −25°C.
- One day at +2 to +8°C.
- Maximum of 6 hours at +15 to +25°C.

General Considerations

Precautions

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

RNA Instability

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. To prevent this instability:

(1) Disrupt cells and stabilize RNA (by inactivation of RNases) as soon as possible after sample collection.
– To obtain good preparations of eukaryotic mRNA, minimize the activity of RNases liberated during cell lysis by using methods that disrupt cells and inactivate RNases simultaneously.

(2) Do not store blood or bone marrow samples for more than a few hours if stabilization is not possible.
– Even in their natural environment within the cell, most mRNAs are extremely unstable.
– The storage of cells in an “artificial” environment results in qualitative and quantitative changes of the mRNA content of the cells.

RNA Stabilization

Disrupting cells in guanidinium lysis buffers is an efficient way to protect against RNase-mediated degradation. RNA lysis buffers that contain guanidinium thiocyanate or guanidinium-HCl consistently yield a high-quality sample.

Rare Cell Detection

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

- Omit cell separation steps to proactively avoid loss of target cell, whenever possible. Particularly tumor cells and micrometastases are heterogeneous. There is no one tumor and the behavior 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, for example, 1 ml or more, should be used for one single RT-PCR reaction. 1 ml of normal human blood contains 4 to 20 µg total RNA which is too much to be used in one RT-PCR reaction. For mRNA, the corresponding amount of 100 to 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 contain many potent inhibitors of the RT-PCR reaction, such as hemoglobin and the anti-coagulant heparin. If the isolated RNA is not diluted for RT-PCR, the efficiency of the purification procedure is extremely important.

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Pretreatment of Reagent

⚠ ***The reagent crystallizes at temperatures <+20°C. Check the solution for the absence of crystals before use, and warm to +37°C if it is not completely dissolved. Thoroughly mix before use.***

1. Label an appropriate container (bottle or tube) with all important information, such as identification, date/exact time of collection, delay between collection and stabilization, type of sample, sample volume, etc.

2. Transfer the 10 volumes of the RNA/DNA Stabilization Reagent for Blood/Bone Marrow into the container per volume of sample, for example, 50 ml per 5 ml blood or bone marrow.

3. Add blood or bone marrow sample and mix vigorously by, for example, vortexing.

4. Ensure that the sample is properly stored until mRNA and or DNA isolation.

⚠ ***Do not store longer than 12 months at −15 to −25°C, 1 day at +2 to +8°C, or 6 hours at +15 to +25°C. For transport, deep freeze the lysates to ≤−25°C.***

Pretreatment of Samples Before mRNA Isolation

1. If lysates were deep frozen to ≤−25°C, thaw samples carefully.

2. Prewarm lysate to +15 to +25°C.
   – Thoroughly mix by, for example, vortexing to ensure that crystallized material is fully dissolved.

3. Do not store the thawed samples at temperatures above +25°C.
   – Review storage conditions in section, **Sample Materials**.

Overview of mRNA Isolation Using the mRNA Isolation Kit for Blood/Bone Marrow

mRNA isolation using the mRNA Isolation Kit for Blood/Bone Marrow includes the following steps. For specific information, see the Instructions for Use of the kit.

1. 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.

2. MGPs are separated by centrifugation or magnetic force and unbound material is removed by washing.

3. Nucleic acids are eluted from the MGPs.
   – At this stage, an aliquot of the nucleic acids can be used for DNA analysis.

4. mRNA is captured from total nucleic acids by using biotin-labeled oligo(dT) and streptavidin-coated magnetic particles (SMPs).

5. SMPs are separated by magnetic force and unbound material is removed by washing.

6. mRNA is eluted after removal of other nucleic acids (DNA, rRNA, tRNA) by washing.

3. Results

Figures 1 and 2 show typical results regarding stability of sample lysates and detection of melanoma cells in bone marrow (model system).

Isolated mRNA Maintains a High Degree of Integrity

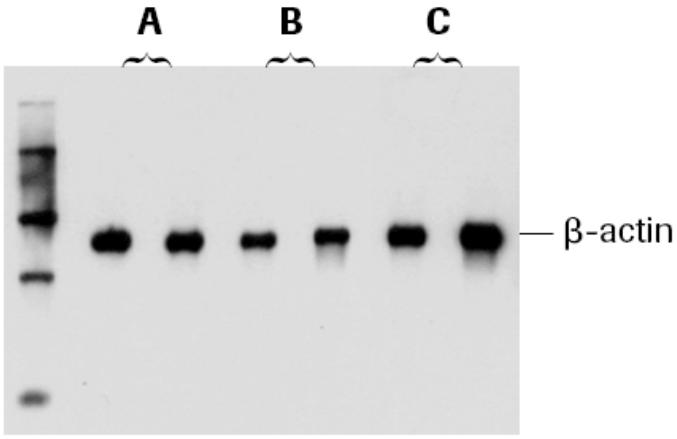

Fig. 1: Six ml of normal human heparinized blood was lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Three aliquots of the lysate corresponding to 2.0 ml blood each were stored at +2 to +8°C (A), +15 to +25°C (B), and −15 to −25°C (C) for 4 days. The mRNA was isolated in duplicate from each sample using the mRNA Isolation Kit for Blood/Bone Marrow. Eluates corresponding to 1 ml of blood were analyzed by northern blotting using a DIG-labeled anti-sense RNA β-actin probe to confirm mRNA integrity.

Bone Marrow is Suitable for Tumor Cell Detection

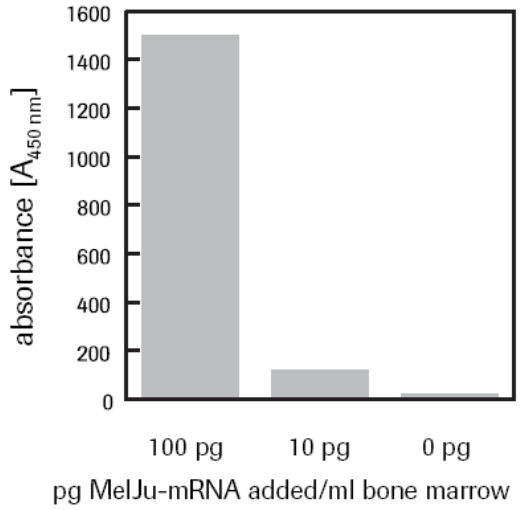

Fig. 2: Three ml of human heparinized bone marrow was lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Three aliquots of the lysate corresponding to 1 ml bone marrow each, were spiked with 100 pg, 10 pg, or 1 pg of melanoma cell line (MelJu) mRNA. The mRNA was isolated from each lysate 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 product was detected by PCR ELISA.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low yield of mRNA, for example, 50 ng/ml of human blood after isolation using the mRNA Isolation Kit for Blood/Bone Marrow.	Lysate was not stored properly before isolation of mRNA.	⚠ ***Do not store lysates more than 12 months at −15 to −25°C, 1 day at +2 to +8°C, or 6 hours at +15 to +25°C.***
	Blood was not stored properly before stabilization.	Storage of whole blood results in continuous loss of mRNA. Do not store unstabilized samples for longer than a few hours.
Integrity of isolated mRNA is not acceptable.	Lysate was not stored properly before isolation of mRNA.	⚠ ***Do not store lysates more than 12 months at −15 to −25°C, 1 day at +2 to +8°C, or 6 hours at +15 to +25°C.***

5. Additional Information on this Product

5.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
mRNA Isolation Kit	1 kit	11 741 985 001
DNA Isolation Kit for Mammalian Blood	1 kit, 25 purifications	11 667 327 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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