

## GeneMATRIX Universal Blood RNA Purification Kit

Kit for isolation of total RNA from human blood and from animal blood samples (mammalian). Applicable for both, fresh and frozen blood samples.

● **Cat. no. E3594**

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<b>Content</b>	<b>25 preps E3594-01</b>	<b>Storage/Stability</b>
Lyse Blood	135 ml	15-25°C
RL	9 ml	15-25°C
RINSE P	90 ml	15-25°C
PSB	6 ml	15-25°C
Proteinase K (20 mg/ml)	0.75 ml	-20°C
Wash DN1	15 ml	15-25°C
Wash RBW	30 ml	15-25°C
DNR	1.5 ml	15-25°C
RNase-free water	3 ml	15-25°C
Homogenization Columns	25	2-8°C
RNA Binding Columns	25	2-8°C
Protocol	1	

# Introductory Notes

**NOTE 1 • Kit Specification.** This kit is designed for isolation of total RNA (longer than 200 bases) from human blood (up to 1.5 ml) or from animal blood (up to 0.5 ml mammalian blood). The kit features a convenient lysis and storage buffer, which, upon collection, immediately stabilizes and preserves total RNA. Since proteins such as RNases are immediately inactivated upon mixing, and RNA degradation as well as gene induction are effectively inhibited, no change in total RNA composition and no loss of RNA will occur in the time between sampling, transportation to the laboratory and subsequent processing of the sample. It is even possible to store blood samples mixed with lysis buffer at -20°C for extended time periods (up to several months). In daily practice, blood samples are collected into vacuum blood tubes containing EDTA and are subsequently mixed with Lyse Blood buffer as soon as possible.

**NOTE 2 • Blood Sample Storage.** Blood samples in Lyse Blood buffer are stable at room temperature for up to 24 hours, at 4°C for up to few days or at -20°C for a few months. The recommended procedure for good working practice is, to keep blood samples stabilized with Lyse Blood buffer at -20°C or below.

**NOTE 3 • Maximum Sample Volume.** One mini column allows purification of RNA from a maximum of 1.5 ml human blood sample. The maximum volume of the column reservoir is 650 µl. The total RNA binding capacity is 125 µg per spin-column.

**NOTE 4 • Column Overloading.** Avoid overloading the mini columns. Overloading results in a significant reduction of both yield and purity and may additionally lead to column-clogging.

**NOTE 5 • Non-Suitable Sample Material.** Blood samples stored at -20°C without prior addition of Lyse Blood buffer are no longer suitable for RNA isolation, due to intrinsic RNA degradation.

**NOTE 6 • β-Mercaptoethanol / DTT.** Contaminating RNases are inactivated by addition of reducing agents capable of disrupting disulfide bonds, such as β-mercaptoethanol (β-ME) or dithiothreitol (DTT). To promote reduction of disulfide bonds, add 10 µl β-ME per 1 ml of buffer RL before use. Upon addition of β-ME, RL buffer remains stable for 1 month. A less toxic but more expensive alternative to β-ME is, to add 10 µl of [1 M] DTT in RNase free water per 1 ml buffer RL before use. DTT is not stable in buffer RL, thus DTT-supplemented RL buffer aliquots must not be stored. Working aliquots of [1 M] DTT stock solution in RNase free water must be stored at -20°C for maintaining stability. To set up a [1 M] DTT stock solution (MW = 154.25 g mol<sup>-1</sup>), dissolve 1.54 g DTT per 10 ml RNase free water and store in aliquots for one-time usage.

**NOTE 7 • Kit Compounds Storage.** Keep all solutions tightly closed to avoid evaporation, resulting in components concentration changes. Store all components of the kit at room temperature with the exception of Proteinase K and Homogenization and RNA Binding columns. Proteinase K should be kept at -20°C. Homogenization and RNA Binding columns should be kept at 2-8°C.

**NOTE 8 • Maintaining Good Working Practice.** To obtain high quality RNA, stick carefully to the protocol provided below. One of the most critical issues during RNA isolation is, to ensure working quickly and with practiced hand. RNA isolation should be performed at room temperature throughout the entire process.

**NOTE 9 • No DNase Digestion Required.** The procedure effectively removes DNA when used for blood sample volumes of up to 1.0 ml of human blood sample or of up to 0.5 ml of animal blood sample (mammalian). DNase digestion is usually not required. In case RNA isolation is performed from large sized blood sample volumes, or if presence of any remaining DNA trace needs to be excluded for formal reasons, perform an optional DNase digestion step (as outlined in Appendix 1, page 9).

**NOTE 10 • Try Using Non-Autoclaved Plasticware.** Autoclaved plastic ware is sterile, but may not be completely RNase- and DNA-free. Neither RNases nor DNA do completely disintegrate at 120°C. Autoclave-borne aerosols are known to transport traces of RNase and of DNA into autoclaved liquids and onto the surface of plastic ware. Especially RNases are known as tough enzymes with the capability for partial refolding at room temperature, even upon autoclave treatment. Do not autoclave mission-critical liquids and plastic ware, especially if autoclaves were previously used for preparation of bacterial agar, for media or for sterilization of lab waste (e.g. old cultures). Use of two separate autoclaves, one reserved for defined buffers and plastic ware only, and one for sterilizing complex media and lab waste, is highly recommended. With high quality plasticware, autoclaving is not required, though: Modern high quality plastic reaction tubes are produced without any human interference, are per default DNA and RNase free, and, when stored clean and dust free, are most often best to use without any autoclave treatment. For convenient storage of plastic tubes at the lab bench, dry-sterilize a set of glass beakers (common preserving jars are perfectly suited for this purpose) and, following cooling, aliquot a freshly opened bag of high quality plastic reaction tubes into each of the sterilized beakers. Close all beakers with the accompanying lid. Always use a clean forceps (not gloved hands or even bare hands) for picking individual plastic reaction bottles during daily lab routine work.

## ***Equipment and reagents to be supplied by the experimenter***

1.  $\beta$  mercaptoethanol [14.3 M] ( $\beta$  ME), or [1 M] Dithiothreitol (DTT) in RNase free water, ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5–2 ml tubes. If the blood sample volume exceeds 500  $\mu$ l – appropriate size plastic tubes for blood lysis and centrifugation after lysis. Optional: vacuum tubes with EDTA for blood collection.

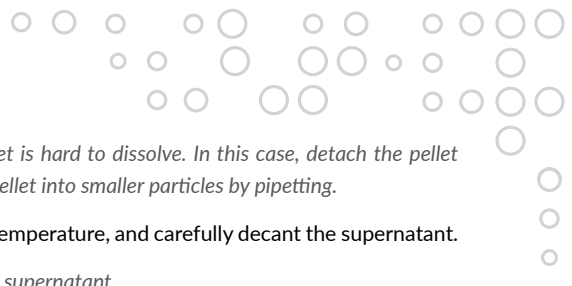


## RNA Isolation from Blood: A Brief Outline of the Protocol

- **Cell Lysis.** Mixing of blood sample with Lyse Blood Buffer results in complete lysis of all blood cells. All proteins, including RNases, are immediately inactivated and denatured. Lyse Blood Buffer stabilizes and protects RNA against RNases and gene induction. Following mixing with Lyse Blood Buffer, the blood sample can either be processed immediately, or else can be frozen and stored for several months. Furthermore, addition of Lyse Blood buffer causes RNA (as well as other lysate components) to precipitate.
- **Homogenization, Removal of DNA.** Follow-up steps include centrifugation (for pelleting of RNA), removal of supernatant and re suspension of the RNA-containing pellet. The re solubilized pellet is subjected to Proteinase K digestion and subsequently transferred to a homogenization spin-column. During homogenization spin-column passage, genomic DNA is sheared and remains bound to the column matrix. RNA remains in solution and is collected with the flow through. Due to DNA removal, viscosity of the RNA-containing flow-through appears reduced.
- **RNA Binding and Washing.** The RNA-containing flow-through is applied to the RNA binding spin-column. RNA molecules get adsorbed to the RNA-binding column matrix with high selectivity. Contaminants – even chemically similar compounds such as polysaccharides as well as remaining traces of DNA – interact only weakly with the matrix and are efficiently removed after two washing steps.
- **Elution.** High-quality RNA is then eluted in RNase-free water.
- **Cutoff Size.** Total RNA longer than 200 bases is recovered with high efficiency. RNA molecules smaller than 200 bases are recovered as well, but with gradually decreasing efficiency. Isolated RNA is ready for downstream applications without the need for ethanol precipitation.

# Main Protocol

1. Add 3 volumes of **Lyse Blood** buffer to a fresh blood sample. Mix by inverting the tube.
  - **Example:** Add 900  $\mu$ l of Lyse Blood buffer to a starting sample volume of 300  $\mu$ l.
  - **Vacuum Blood Tubes.** Blood may be collected into vacuum blood tubes containing EDTA. Add an appropriate volume of Lyse Blood buffer as soon as possible.
  - **Sample Storage.** When preparing a blood sample for freezing with Lyse Blood buffer – after mixing, prior to freezing -, allow the sample to incubate for approximately 30 min at room temperature.
  - **Sample Volume.** Do not use less than 0.2 ml or more than 1.5 ml of human blood. Typically, ~ 2  $\mu$ g of total RNA is isolated from 0.5 ml of a blood sample originating from a healthy person. For animal blood samples, do not use more than 0.5 ml of blood. Typically, ~ 5  $\mu$ g of total RNA is isolated from 0.1 ml of a mouse blood sample or ~ 20  $\mu$ g of total RNA is isolated from 0.5 ml of a rat blood sample.
  - **Large Volume Samples.** When processing larger volumes of blood samples, choose one of two options: Either apply two ethanol-supplemented lysates (step 13 of the protocol) from two homogenization spin columns subsequently (one after another) to one single RNA binding column. Else, see the method outlined in the appendix, page 8.
  - **Non-Suitable Blood Samples.** Do not attempt to isolate RNA from frozen blood samples in case Lyse Blood buffer was not already mixed with the blood sample prior to first frozen storage.
2. Leave at room temperature for a minimum 15 min to achieve complete cell lysis and to allow for RNA precipitation. Mix a few times by inverting the tube.
  - **-20°C Samples.** For blood samples frozen with Lyse Blood buffer (-20°C or below), thaw samples at room temperature. Do not heat. During thawing, mix the sample a few times by inverting the tube and, upon completion of the thawing process, equilibrate the samples at room temperature for 15–30 min.
  - **+4°C Samples.** For blood samples supplemented with Lyse Blood buffer, and subsequently stored in a refrigerator (+4°C), allow the liquid sample to equilibrate at room temperature for 15–30 min prior to isolation.
3. Centrifuge at 1500 x g for 8 min at room temperature, and carefully decant the supernatant.
  - Carefully pipet to remove the remaining supernatant.
4. Add two volumes of **RINSE P** solution to the pellet. Mix thoroughly by vigorous vortexing or pipetting.
  - **Example:** Add 600  $\mu$ l of RINSE P buffer to a starting blood volume of 300  $\mu$ l.

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- **Pellet Solubilization.** Sometimes the pellet is hard to dissolve. In this case, detach the pellet from the bottom of the tube and shear the pellet into smaller particles by pipetting.
5. Centrifuge at 1500 x g for 8 min at room temperature, and carefully decant the supernatant.
    - Carefully pipette to remove the remaining supernatant.
  6. Suspend the pellet in 200 µl of **PSB** solution. Mix thoroughly by pipetting.
    - **Pellet Solubilization.** Sometimes the pellet does not dissolve well. It is important to shear the pellet into small pieces. Pellet breakup greatly simplifies the dissolution of the precipitate in RL buffer during the next step.
  7. Add 300 µl **RL** buffer to the suspended pellet. Mix thoroughly by pipetting to dissolve suspended precipitate.
    - Ensure that either β-ME or DTT is added to buffer RL (see page 3 note 6).
    - Upon addition of RL buffer, carefully dissolve the pellet in PSB buffer by repeated pipetting until complete dissolution. Should, despite all efforts, some fine particles still remain undissolved, continue with the next step (step 8, Proteinase K digestion) and try dissolving the yet non-soluble sediment by thorough pipetting in step 10.
  8. Add 25 µl of **Proteinase K**. Mix by pipetting or vortexing the tube.
  9. Incubate at 50°C for 10–15 min.
  10. Following digestion thoroughly mix sample by pipetting, and dissolve any remaining precipitate. Add 80 µl of ethanol (96–100%) to the sample. Mix thoroughly by pipetting. Do not centrifuge.
  11. Carefully transfer the sample to the **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
    - Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.
  12. Add 200 µl 96–100% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
    - A precipitate may form after addition of ethanol.
  13. Apply up to 600 µl of the sample, including any precipitate, to the **RNA binding spin-column** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
  14. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
  15. Add 500 µl of **Wash DN1** buffer and centrifuge at 11 000 x g for 1 min.
-

16. Remove the spin-column, discard the flow-through and stick the spin column back onto the receiver tube.
17. Add 600  $\mu$ l of **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
18. Remove the spin-column, discard the flow-through and stick the spin column back onto the receiver tube.
19. Centrifuge at 11 000 x g for additional 1 min to remove any residual wash buffer.
20. Place spin-column onto a fresh receiver tube (1.5–2 ml) and add 40–60  $\mu$ l **RNase-free water** directly onto the membrane.
21. Centrifuge for 1 min at 11 000 x g.
22. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Isolated RNA can be stored either at 2–8°C (preferred) or at -20°C. Avoid multiple freezing and thawing of RNA, since mRNA is susceptible to freezing-thawing. Prepare RNA aliquots.

## Isolation of Blood Samples Exceeding 0.5 ml in Volume

1. Either perform lysis of large volume blood samples in large reaction tubes, respectively. Alternatively, divide the blood sample into max. 0.5 ml aliquots. Subdividing the sample allows to perform lysis by using 2 ml tubes and standard microcentrifuges, and overcomes the requirement for large reaction tubes and for centrifuges with large rotors. In either case, add 3 volumes of Lyse Blood Buffer to each sample. Example: If 800  $\mu$ l of blood is divided in two portions of 400  $\mu$ l each, transfer both aliquots in two separate 2 ml tubes and add to each aliquot 1.2 ml of Lyse Blood buffer.
2. For each tube, proceed with sample preparation as outlined in the main protocol (incubation for 15 min at RT, centrifugation for 8 min @ 1500 x g, pellet isolation) until step # 4.
3. Suspend the first pellet and the second pellet separately in 0.8 ml each of RINSE P solution. Centrifuge both solubilized pellets at 1500 x g for 8 min at room temperature, keep the newly formed pellet and discard the supernatant, as outlined in step 5 of the main protocol.
4. Following centrifugation, dissolve the first pellet in 200  $\mu$ l buffer PSB until the pellet has dissolved to completeness. Transfer the RNA-PSB solution to the second pellet and continue to dissolve the second pellet completely. Both pellets are now dissolved in a total volume of 200  $\mu$ l buffer PSB. Continue with step 7 in the main protocol.



# Appendix

## RNA Purification with Optional On-Column DNase Digestion

**NOTE 1 • Digestion Follows First Washing Step.** Use this protocol after having performed the first washing step with buffer Wash DN1 (see Point 15 in the main protocol).

**NOTE 2 • Use solely buffer DNR for performing on-column DNase digestion.** DNR buffer ships with the kit. Other DNase buffers are not compatible with this on-column DNase digestion step.

**NOTE 3 • RNase-free DNase I.** For obvious reasons, use only RNase-free DNase I.

**NOTE 4 • Use high quality DNase I.** DNase I is not supplied with this kit. Since there are obviously huge quality differences between RNase free DNase: Choose only high quality DNase I that does not show any signs of interference with RNA, even during prolonged incubation periods. High quality RNase free DNase I is available as a separate product (Cat. no. E1345).

**NOTE 5 • Preparation of DNase I solution.** Prepare DNase I solution before starting this procedure. Add 1–2 U (Kunitz) of DNase I per 50 µl DNR buffer. Do not add more than 2 µl of DNase I solution per 50 µl of DNR buffer. For lyophilized DNase I: Dissolve solid DNase I in the storage buffer (50 mM Tris-acetate pH 7.5, 10 mM CaCl<sub>2</sub> and 50% v/v glycerol) in a concentration of 1–2 U/µl (Kunitz) and then add 1 µl of DNase I per 50 µl of DNR buffer.

**NOTE 6 • Sensitivity of DNase I.** DNase I is sensitive to physical denaturation. Be careful not to mix DNase too vigorously.

1. Upon completion of step 15 (with washing buffer **Wash DN1**) and removal of any residual wash buffer by centrifugation, remove the spin-column, discard the flow-through and stick the dry column back onto the receiver tube.
2. Add 50 µl of **DNR** buffer, with **DNase I** added, directly onto the membrane and place on the benchtop at room temperature for 10 min. Do not centrifuge.
  - Ensure that DNase I has been added to buffer DNR. See note 4 above.
3. Add 400 µl of **Wash RBW** buffer and spin down at 11 000 x g for 1 min.
4. Remove the spin-column, discard the flow-through and stick the column back onto the receiver tube.
5. Continue with the **Wash RBW** step in the standard protocol (see Point 17) and follow the standard protocol to the end.

# Quality Control of Isolated RNA

Quality of isolated total RNA is routinely assessed

– **for purity:** By spectrophotometric measurement

Criteria for high quality RNA:

- A260/A280 ratio 1.8–2.1 (high quality range)
- RNA concentration:  $A_{260} = 1 \approx 40 \text{ ng} / \mu\text{l} \times \text{sample dilution factor}$


– **for physical integrity:** By agarose gel electrophoresis (1–1.5% [w/v]) – quick check.

Criteria for high quality RNA:

- Distinctly visible, prominent and sharp 28S and 18S rRNA bands, no visible band retardation (indicative for RNase action),
- 28S/18S rRNA band intensity ratio is  $\geq 2:1$ ,
- the “smear” appearing among the prominent 28S and 18S rRNA bands represents mRNAs of various sizes and spans the region between both prominently visible bands (the “smear” should not concentrate in the small-sized-RNA region),
- no enrichment of small or very small RNAs is observed ( $<100 \text{ nt}$ ), which would be indicative for RNA degradation,
- no visible band appears at  $\sim 20 \text{ kb}$  (indicative for contamination by genomic DNA),
- total RNA appears in distinct, but tissue-specific patterns.

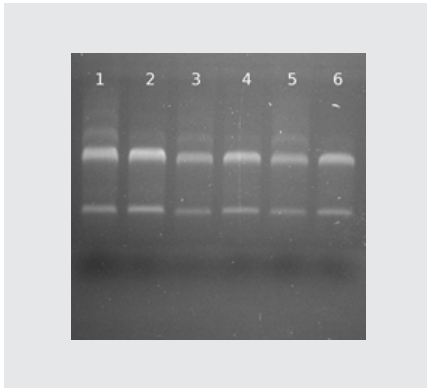
**NOTE 1 · ssRNA Migration Behaviour.** 28S rRNA and 18S rRNA are single-stranded molecules, and, due to their biological function, form extensive, stable secondary structures. Both rRNAs will therefore migrate faster through a non-denaturing agarose gel, as compared to a dsDNA molecular weight standard. A direct size comparison of rRNAs to dsDNA marker is thus not possible. However, both ribosomal RNA bands are easily and unambiguously identified due to their prominent intensity and appearance. In contrast, precise RNA size comparisons would require much more work-intensive denaturing polyacrylamide gel electrophoresis. For the sole application within a mere routine quality control, the extra workload for preparing and running PAGE gel electrophoresis is, in our eyes, not justified. PAGE gel electrophoresis would add no surplus of relevant information in this application.

**NOTE 2 · 28S/18S rRNA Band Intensity Ratio > 2:1.** In living cells, 28S rRNA and 18S rRNA, respectively, are always present in a 1:1 stoichiometric ratio. Since 28S rRNA is more than double the size of 18S rRNA (for Homo sapiens: 28S rRNA  $\sim 5000 \text{ nt}$ , 18S rRNA  $\sim 1900 \text{ nt}$ ; similar values apply throughout the animal kingdom): The gel band representing 28S rRNA is supposed to appear with more than double of the intensity as compared to its 18S rRNA counterpart. Thus, a 28S / 18S rRNA ratio of  $<2$  and / or visible band retardation are indicative for RNA degradation.

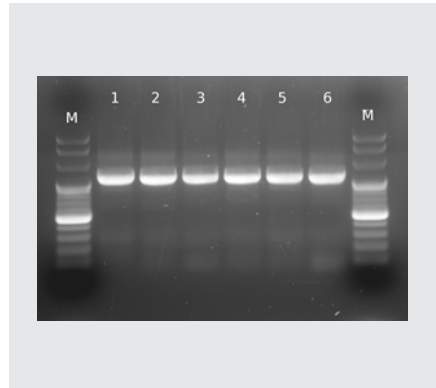


**NOTE 3 • Additional Optional Quality Controls.** Additional optional quality controls include (1) checks for DNA contamination (PCR of non-reverse transcribed RNA must not result in PCR amplicon formation; perform check against a positive control with genomic DNA) and (2) of target gene amplificability following reverse transcription. A known-to-work PCR assay for a human DNA target gene is the assay for  $\beta$ -Actin, chromosome 7, a housekeeping gene (Adjaye et al. 1999, Gene 237: 373–383). PCR amplicon size differs between genomic DNA (565 bp) and cDNA (470 bp). Primer pair:  $\beta$ -Act sense 5'-CGG ATG TCC ACG TCA CAC TT-3';  $\beta$ -Act anti 5'-GTT GCT ATC CAG GCT GTG CT-3'; PCR conditions: 95°C / 120 s – 35 x [94°C / 30 s – 60°C / 30 s – 72°C / 120 s] – 72°C / 600 s.

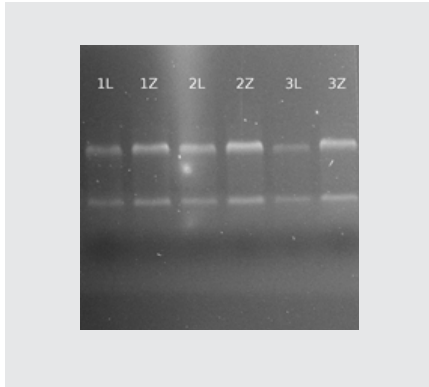
Figures 1a to 2b (page 12 to page 13) illustrate an example quality control for total RNA isolated from fresh and from frozen human blood samples.



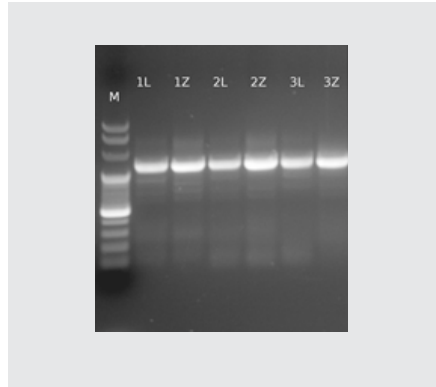
**Fig. 1a. Fresh human blood RNA isolation results. Agarose gel electrophoresis of isolated total RNA.** Isolation performed two hours after blood collection. Volume of blood samples per preparation was 0.5 ml each, total elution volume was 60  $\mu$ l. 20  $\mu$ l per lane (~ 1/3 of isolated RNA) was loaded per lane (Note: When performing routine controls, usually less volume is required, e.g. ~ 1/5 of isolated RNA). Prominently visible bands represent 28S and 18S rRNA, respectively. Isolated RNA is of visible high quality, since 28S/18S band intensity ratio is clearly  $>2$ . No visible signs for RNA degradation at the lower end of the gel. No visible band appears at ~20 kb, which would be an indicative for genomic DNA contamination. RNA quality control passed successfully.



**Fig. 1b. Gen ARG I RT-PCR results.** RT-PCR was performed using EURx OneStep RT-PCR kit (EURx cat. no. E0803) and 4  $\mu$ l of RNA isolated as described in Fig. 1A per one reaction was used. The RT-PCR mixture was loaded on an agarose gel. M – Perfect™ 100 bp DNA Ladder (EURx cat. no. E3134).



**Fig. 2a. Stored human blood RNA isolation performed 5 days after blood collection. Agarose gel electrophoresis of isolated total RNA.** Blood with the addition of Lyse Blood buffer stored in refrigerator (L) or in a freezer (Z). Volume of blood – 0.5 ml per preparation. Elution volume was 60  $\mu$ l. 20  $\mu$ l per lane (~ 1/3 of isolated RNA) was loaded per lane. Same visual RNA quality as for Fig. 1 a.



**Fig. 2b. Gen ARG I RT-PCR results.** RT-PCR was performed using OneStep RT-PCR kit (EURx cat. no. E0803) and 4  $\mu$ l of RNA isolated as described in Fig. 2a was used per reaction. RT-PCR reactions were loaded on agarose gel. L – indicates blood samples stored with the addition of Lyse Blood buffer in refrigerator, while Z indicates samples stored in a freezer. M – Perfect<sup>TM</sup> 100 bp DNA Ladder (EURx cat. no. E3134).

# Safety Information

## Lyse Blood

### Warning



**H315** Causes skin irritation.

**H319** Causes serious eye irritation.

**H400** Very toxic to aquatic life.

**P280** Wear protective gloves/protective clothing/eye protection/face protection.



**P273** Avoid release to the environment.

**P305+P351+P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

**P302+P352** If on skin: wash with plenty of water.

**P332+P313** If skin irritation occurs: get medical advice/attention.

**P337+P313** If eye irritation persists: get medical advice/ attention.

## Proteinase K

### Danger



**H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled.

**P261** Avoid breathing vapours/spray.

**P304+P340** If inhaled: remove person to fresh air and keep comfortable for breathing.

**P342+P311** If experiencing respiratory symptoms: call a poison center or doctor/ physician.

## RL

### Warning



**H302+H332** Harmful if swallowed or if inhaled.

**H412** Harmful to aquatic life with long lasting effects.

**P273** Avoid release to the environment.

**P301+P312** If swallowed: call a poison center/ doctor/... if you feel unwell.

**P304+P340** If inhaled: remove person to fresh air and keep comfortable for breathing.

**EUH032** Contact with acids liberates very toxic gas.

## Wash RBW

### Danger



**H225** Highly flammable liquid and vapour.

**H319** Causes serious eye irritation.

**P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.



**P280** Wear protective gloves/protective clothing/eye protection/face protection.

**P305+P351+P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

**P403+P235** Store in a well-ventilated place. Keep cool.

**P337+P313** If eye irritation persists: Get medical advice/ attention.

○ ***The GeneMATRIX Universal Blood RNA Purification Kit is a specifically designed spin-column system for rapid isolation of total RNA from fresh or frozen human blood as well as from animal blood samples.***

Only a minimum of hands-on time is required at the sampling site: Blood samples are mixed with a specifically designed lysis buffer, which, upon addition, immediately inactivates any protein, including RNases. Thus, it is possible to conduct blood sampling by personnel lacking any molecular biological background. Followed by transport of the non-perishable sample to dedicated laboratories, further processing and analysis of stored blood samples is then conducted by skilled personnel.

Purified RNA is of high quality, is DNA-free as well as free of contaminants, such as heparin, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others. Rock-solid GeneMATRIX technology forms the basis for efficient recovery of pure RNA.

○ **GeneMATRIX is synonymous for a family of synthetic, new generation, nucleic acid binding membranes. The GeneMATRIX membrane family has gained fame for two striking features: First, for their extraordinary high binding capacity, allowing to isolate nucleic acids with optimal yield.**

Second, for their remarkably high specificity. Even compounds of pronounced chemical similarity such as DNA, RNA and polysaccharides are easily differentiated amongst each other by the selectivity of these highly optimized matrices. This feature allows to isolate highly pure nucleic acids, that remain to work reliable even after being subjected to extended storage periods (years). Or, directly upon isolation, when used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA-synthesis, hybridization, etc ...

We take great care to fine-tune all components and variables of the nucleic acid purification system towards each other – a multi-parameter optimization. All matrices ship conveniently pre-packed in a ready-to-use spin-column format. Spin columns are specifically constructed for precise adjustment of liquid flow-through rate to optimal values. Novel

binding and washing buffers are developed to take full advantage of GeneMATRIX unique capacity, resulting in isolation of biologically active, high-quality nucleic acids. And, last not least, a lot of time and efforts went into development of the various GeneMATRIXes, thus providing a platform of unique chemical composition. High and continuous reproducibility of matrix performance is always warranted, since component preparation as well as stringent quality control is entirely performed in-house at EURx Ltd.

Whatever your experience with nucleic acids isolation kits may look like, most likely you will encounter a difference with GeneMATRIX. And, we are so much convinced, you'll love it. Enjoy.



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