



For *in Vitro* Diagnostic Use

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# RNA-medium

Medium for stabilization and preservation  
of blood cell mRNA

## Instruction Manual

# AmpliSens®



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## 1. INTENDED USE

**RNA-medium** is a medium intended for taking, transportation, and storage of whole blood samples with simultaneous stabilization of cell mRNA and subsequent extraction of total RNA. Phenol extraction of RNA according to Chomczynski or its modifications based on the use of RIBO-zol-A and RIBO-zol-D nucleic acid extraction kits manufactured by CRIE are recommended.

## 2. PRINCIPLE

The first step of many molecular studies of mRNA is collecting whole blood. The main problem of these studies is the instability of cell RNA that degrades rapidly within a few hours. Moreover, the amount of some RNA types in collected blood increases *in vitro* due to gene induction. Both degradation and induction may lead to inadequate estimation of the RNA level *in vivo*.

RNA-medium includes an agent that ensures *in vitro* stabilization of the *in vivo* expression profile by suppressing RNA degradation and minimizing gene induction *in vitro*.

## 3. CONTENT

**RNA-medium** is produced in 1 form:

RNA-medium **REF** 981-CE.

RNA-medium includes:

| Reagent    | Description            | Volume (ml) | Quantity |
|------------|------------------------|-------------|----------|
| RNA-medium | colorless clear liquid | 100         | 1 vial   |

## 4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat.
- Disposable polypropylene tubes
- Automated pipette (adjustable).
- Sterile pipette tips with aerosol barriers.
- Desktop microcentrifuge.
- Deep-freezer for  $\leq -16$  °C.
- Refrigerator for 2–8 °C.
- Tube racks.
- Waste bin for used tips.

## 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a reagent after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in

accordance with appropriate biosafety practices.

- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.

## 6. SAMPLING AND HANDLING



Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**RNA-medium** is intended for transportation and storage of whole blood samples.

### Freeze-thawing

Keep tubes in the upright position when freezing.

Samples placed in RNA-medium can be stored at  $\leq -16$  °C. To do this, incubate the samples at  $\leq -16$  °C for 24 h and then store at  $\leq -68$  °C.

To thaw the samples, incubate the tubes in the upright position at room temperature for about 2 h.



Do not thaw samples at temperature above 25 °C

If the samples were not incubated at room temperature for 4 hours before freezing, prior to RNA extraction they should be additionally incubated for at least 2 h after they reach room temperature.

## 7. PROTOCOL

### Recommended test formats

| Tube | Volume, ml  |            |
|------|-------------|------------|
|      | Whole blood | RNA-medium |
| 1.5  | 0.35        | 1.05       |
| 2.0  | 0.5         | 1.4        |
| 5.0  | 1.25        | 3.5        |
| 10.0 | 2.5         | 6.9        |



Prior to taking blood, make sure that a tube with RNA-medium was stored at 18–25 °C and it does not contain crystals. If crystals were formed then incubate the tube at room temperature until the crystals disappear

1. Collect a blood sample in a disposable sterile tube with EDTA and mix. Just after that transfer the required amount of collected blood in a tube with RNA-medium.
2. Immediately mix the tube content by turning the tube over 8-10 times.

- Incubate the tube in the upright position at room temperature for at least 4 h and at most 72 h before placing in a refrigerator (at 2–8 °C) or freezer (at ≤–16 °) or before RNA extraction.
- Prior to RNA extraction, centrifuge the tubes at 5,000 g (for example, at 13400 rpm in the case of Eppendorf minispin and 1.5-ml (2.0-ml) tubes). Then, extract RNA from the pellet.

If RNA is extracted according to Chomczynski, the pellet may not disappear until phenol is added. This is acceptable; however, when phenol is added, dissolve the pellet under thorough stirring.

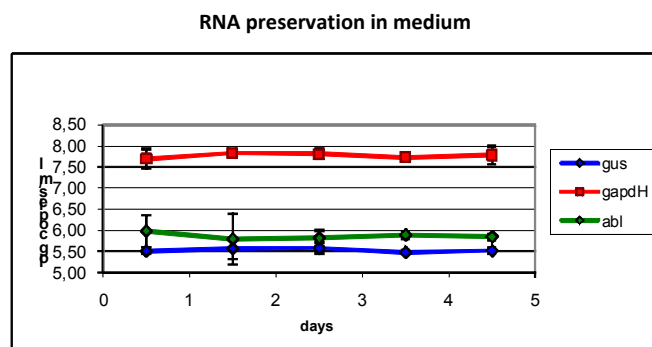
## 8. STABILITY AND STORAGE

**RNA medium** is to be stored at 18–25 °C when not in use. RNA-medium is stable until the expiration date on the label.

## 9. SPECIFICATIONS

### 1. Analytical performance

If storage conditions are strictly followed, the expression profile of cell mRNA is stable at 2-8 °C for 5 days, at room temperature for 3 days, and at ≤–16 °C for 6 months.



Preservation of mRNA and stability of expression profile were assessed on whole blood samples placed in the RNA-medium. The test tubes were stored at room temperature for 4.5 days. RNA was extracted every day in triplicate. The expression profile of three human genes, β-glucuronidase (*gus*), glyceraldehyde 3-phosphate dehydrogenase (*gapdH*), and Abelson (*abl*), was evaluated by using quantitative RT-PCR. The diagram above shows the mean logarithm of mRNA copy concentration in one ml of extracted RNA and standard deviations (at  $p = 0.95$ ).

## 10. REFERENCES











- Chomczynski P. and Sacchi N. Anal.Biochem 1987,V.162., P.156-159.

- Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics” developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

## 11. QUALITY CONTROL

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485-Certified Quality Management System each lot of **RNA-medium** has been tested against predetermined specifications to ensure consistent product quality.

## 12. EXPLANATION OF SYMBOLS

|   |                                    |  |   |
|---|------------------------------------|--|---|
|    | Manufacturer                       |   | Temperature limitation                                      |
|    | Use by                             |   | Batch code  |
|    | For <i>in Vitro</i> Diagnostic Use |   | Version   |
|   | Catalogue number                   |  | Caution, consult accompanying documents                     |
|  | Contains sufficient for <n> tests  | <b>CRIE</b>  | Central Research Institute of Epidemiology (Moscow, Russia) |
|  | Consult instructions for use       |  |   |