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RNaseLag

Stabilization reagent for gene expression profile in harvested samples

By ROJE

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ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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Storage

Shipment condition is checked by ROJETechnologies. After arrival, RNaseLag should be kept dry at room temperature (15-25 °C). When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

Notice that, RNaseLag is intended for molecular biology applications not for diagnostic use. We recommend all users to study RNA experiments guideline, before starting their work.

Guarantee & Warranty

ROJETechnologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our technical support team. If problem is due to manufacturing process, ROJE team will replace the Kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell this kit or any components. For information about out licensing or distributors contact ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Quality Control

RNaseLag is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at www.rojetechnoloes.com.

Description

To stop changes in the gene expression pattern, nonspecific RNA degradation and transcriptional induction in all reliable quantitative gene expression analyses, it is necessary to stabilize RNA in biological samples immediately, after harvesting. RNaseLag reagent makes it possible for researchers to postpone RNA isolation for even months after sample collection without losing RNA integrity.

Protocol RNA Stabilization in Harvested Animal Tissues

- Cut the animal tissue sample into slices less than 5mm thick, as quickly as possible.
- Completely immerse the tissue pieces in the collection vessel containing RNaseLag.

  Note: Make sure to use the appropriate volume of RNaseLag, so weight your sample before starting the procedure and use 10µl RNaseLag per 1mg of tissue.

- The sample is ready for archival storage at conditions shown in Table 1.
- After storage, for RNA isolation continue with appropriate protocol for the chosen sample type.

Table 1. Storage conditions and procedures after RNaseLag treatment.

Protocol	Storage condition
Incubate the prepared sample (in RNaseLag) for up to 4 weeks at 2–8°C.	**2–8°C**
Incubate the prepared sample (in RNaseLag) for up to 7 days at 15-25°C.	**15-25°C**
Incubate the prepared sample (in RNaseLag) for up to 1 days at 37°C.	**37°C**
First incubate the prepared sample (in the RNaseLag) overnight at 2–8°C. Then transfer it to –20°C for storage.	**–20°C**
First incubate the prepared sample (in the RNaseLag) at 2–8°C. Then remove the tissue from the reagent, and transfer it to –80°C for long storage.	**–80°C**

Protocol RNA Stabilization in Harvested Bacterial Cells

Process

- Calculate the required volume of bacterial culture (refer to Appendix 7).
- Add 2 volumes of RNaseLag into a tube

- Add 1 volume of bacterial culture to the tube. Mix by vortexing for 5 sec. Incubate at room temperature (15–25°C) for 5 min.
- Centrifuge for 10 min at 4000 rpm at universal centrifuge.

  Note: Sometimes the pellet is too clear to be recognized, it is due to RNaseLag treatment, and will not affect the ongoing process.

- Decant the supernatant.
- Pellets can be stored at –20 to –30 °C for up to 2 weeks or at –70 °C for up to one month. For RNA isolation, thaw pellets at room temperature (15–25 °C) and proceed the appropriate RNA isolation protocol.

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
RNA Degradation	Stabilized old tissue	• Use fresh tissue. Immediately after harvesting sample, submerge sample in RNaseLag.
	Not appropriate amount of RNaseLag	• Weight your sample before starting the procedure and use 10 µl RNaseLag per 1mg of tissue.
	Too thick sample for stabilization	• Cut samples into slices less than 5 mm and then submerge in RNaseLag.
	RNase contamination during RNA purification	• RNases can be introduced during RNA isolation. Refer to appendix 1 for more information.
	Storage duration is exceeded in RNaseLag	• Refer to Table 1.

Ordering Information

Category	Product Name	Cat No.	Size
RNA Care	RNaseLag	RN983016	50ml

Technical Assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team is composed of highly trained, experienced scientists; who can troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select a suitable product.

- Contact our technical support at any time by selecting one of these ways:
- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website (www.ROJETechnologies. com)
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Appendix 1: Handling RNA

RNA is highly sensitive to degradation due to the ubiquitous presence of RNases in the environment. unlike DNases, which require metal ions for activity, RNases do not require cofactors for their enzymatic activity and can maintain activity even after prolonged boiling or autoclaving. Since RNases are active enzymes, and are too hard to be deactivated, do not use any plasticware or glassware without first removing possible RNase pollution. Special precautions should be taken when working with RNA. All reagents and equipment must be specially treated to inactivate RNases prior to use.

The following are some tips and techniques to remember when working with RNA:

General Tips

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory mobilizations. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
- Treat surfaces of benches with commercially available RNase inactivating agents such as RNZO (Order by cat No RN983018 and RN983019).
- Treat non-disposable glassware and plasticware before use, to ensure that it is RNase-free. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA, and RNase-free water.

- Use disposable RNase-free plasticware to decrease the possibility of sample contamination.
- Keep purified RNA on ice when aliquots are pipetted for downstream applications.
- Glassware should be immersed in freshly prepared %0.1 (v/v) DEPC in water or ethanol for several hours, followed by draining and autoclaving.

Appendix 2: RNA Storage Condition

RNA samples are commonly stored at −20 °C or −80 °C, eluted in RNase-free water. Under these conditions, RNA is not degraded after 1 year.

RNA Quality

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of RNA using UV absorption, by a spectrophotometer or Nanodrop. For pure RNA, A260/A280 ratios should be around 2.1. If the ratio is noticeably lower in either case, it may indicate the presence of protein or other contaminants that absorb strongly at or near 280 nm. High A260/A280 purity ratios are not indicative of an issue.

Appendix 3: RNA Integrity

The integrity of RNA molecules is important for experiments that try to reflect the snapshot of gene expression of RNA isolation. The least expensive method for checking RNA integrity is to run the RNA on a %1 DGE agarose gel and examine the ribosomal RNA (rRNA) bands. The upper ribosomal band (28S in eukaryotic cells and 23S in bacterial cells) should be about twice the intensity of the lower band (18S in eukaryotic cells and 16S in bacterial cells) and should be sharp and tight.

Denaturing agarose gel electrophoresis

Formaldehyde is used to keep the RNA from denaturation, and when RNA is heated in the presence of formaldehyde all secondary structures are eliminated. Therefore, gel electrophoresis of RNA in agarose gels containing formaldehyde provides a good denaturing gel system. Formaldehyde is a potential carcinogen. Take appropriate safety measures and wear gloves when handling.

Gel preparation

- Prepare %1.2-2 gel with TAE 1x buffer (%5 formaldehyde).
- Pour the gel using a comb that will form wells large enough to accommodate at least 20 µl.
- Assemble the gel in the tank.

- Use TAE 1x buffer (%5 formaldehyde) as the tank buffer.

RNA sample preparation

- Add 10 µg of purified RNA to 6 µl of ROJE sample buffer (in RNA Loading Set, order by cat No LD983007) and incubate at 70°C for 3min.
- Add 3 µl Hinari to the mixture of RNA and sample buffer and mix well by pipetting.
- Then run the mixture in the prepared gel.

Electrophoresis

Load the gel and electrophorese at 5-6 V/cm until the loading dye at least has migrated 2/3 the length of gel.

Results

Visualize the gel on a UV transilluminator. The 28S and 18S ribosomal bands should be sharp and intense. The intensity of the 28S band should be twice more than the 18S band. The following Figure shows different RNA samples, isolated by RNJia kit and run by DGE.

Note: If DNA contamination is present, the high molecular weight smear will be appeared above the 28S band.

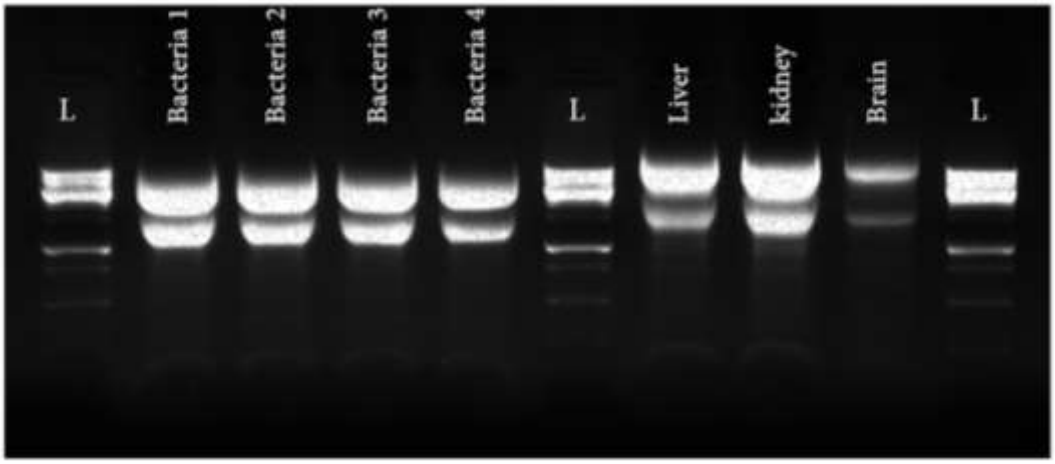

Figure 1. Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10 µg RNA was pretreated by ROJE Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

Appendix 4: DNA Contamination

Different methods exist for monitoring genomic DNA contamination, for example, using OD measurement or via agarose gel electrophoresis. Nevertheless, genomic DNA traces which are not detectable by the above methods, can produce nonspecific results in sensitive downstream

applications. The co-amplification of cDNA and contaminating genomic DNA produce one or more additional PCR fragments that can be traced via agarose gel electrophoresis.
strategies to solve the problem:

- Design PCR primers for span exon boundaries, which includes one or more intronic sequence motifs in the genomic DNA, results in amplification of cDNA template.
- Design a minus control reaction with reverse transcriptase. In that specific reaction, instead of the reverse transcriptase enzyme, water is added to the reaction mixture. PCR product in this control reaction indicate genomic DNA contamination.

Appendix 5: Protein Isolation by RNall Kit

RNall kit is specified for RNA isolation. However, protein can be extracted simultaneously. The denatured protein can be used for SDS-PAGE, western blotting, 2D gel electrophoresis and etc.

Equipment to be supplied by user

- Centrifuge
- Acetone
- Ethanol, molecular biology grade

Process

- Prepare cell lysate (appropriate to sample type) and centrifuge it through a HiPure DR Column, as described in all protocols.
- Do not discard the flow-through. Transfer it to a new clean microtube and Add 4 volumes of ice-cold acetone to it.
- Incubate at -20°C for 30min.
- Centrifuge for 10min at 14000 rpm in a benchtop centrifuge. Discard the supernatant.
- Wash the pellet with 100 µl ice-cold ethanol and air-dry.

  Note: For easier resuspension, do not over dry the pellet.
- Resuspend the pellet in the appropriate buffer according to downstream reaction.

Appendix 6: Convert RPM to RCF (centrifuge)

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$\boldsymbol{RPM} = \sqrt{\frac{\boldsymbol{RCF}}{(\mathbf{1.118} \times \mathbf{10^{-5}})(\boldsymbol{r})}}$$

Where **RCF** = required gravitational acceleration (relative centrifugal force in units of g); **r** = radius of the rotor in cm; and **RPM** = the number of revolutions per minute required to achieve the necessary g-force.

Appendix 7: Cell Count by a Hemocytometer

Sample preparation

First, resuspend the cell in flask, and then quickly remove your aliquot of cells. For a cell culture growing in a Petri dish, you need to resuspend the cells growing on the bottom of the dish by gently using a pipette to remove cells and media from a dish and then gently expelling them back into the dish. This aspiration of the contents of the Petri dish should be completed several times, each time expelling the cells and media while moving the pipette across the bottom of the dish to gently discharge cells growing on the entire surface. After resuspension, the aliquot is quickly removed from the vessel before the cells settle to the bottom again. For cell culture applications cells are often stained with Trypan Blue so that dead cells can be distinguished from live cells. For example, a 0.5ml suspension of cells would be removed from the Petri dish and mixed with 0.5ml Trypan Blue solution in an Eppendorf or small test tube. Trypan Blue is a stain that selectively stains dead cells.

Loading Sample

Load your sample quickly and smoothly. It is important to handle the hemocytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide. Always clean the slide before you load the sample by rinsing the slide and cover slip with %70-95 ethanol. Air-dry or gently wipe the slide and cover slip with lens paper. Place the clean and dry slide on your work surface and place the coverslip on top to cover the reflective surfaces. If you have diluted your cells in a test tube, invert the tube several times to resuspend the cells. Using a micropipette, quickly and smoothly without interruption, add 10 µl of your cell suspension (or 1 drop from a transfer pipette) to the V-shaped groove on each side of the hemocytometer. If your sample moves into the gutters, you may not have loaded the sample in the correct location or you may have used too large of an aliquot.

Estimating cell density

Count all of cells within each of the four large quadrants in the four corners of each counting chamber on the hemocytometer (see Figure 2). Count all of the cells within each quadrant except those on the far-right edge and lower bottom edge.

Calculate the cell density by this formula:

Average number of cells × dilution factor× 10^4

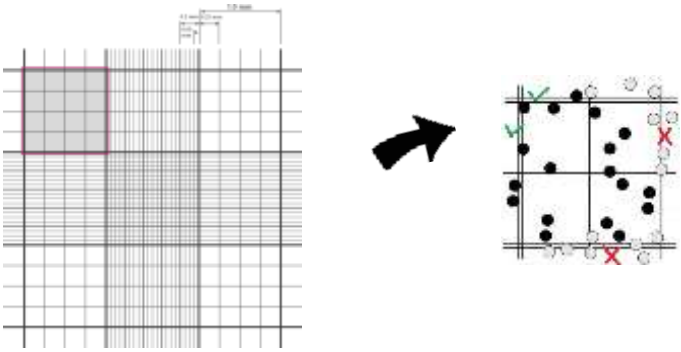

Figure2. Cell counting with hemocytometer

Factory address

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130- phone: +982191070705

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