ROJETECHNOLOGIES

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RNZO

RNase Decontamination Solution

By ROJE

Edition, 01/2022

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.

Contents

Storage

Shipment condition is checked by ROJETechnologies. After arrival, RNZO 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, RNZO 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

MiRJia Kit 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

RNZO reagent is used to remove RNase contamination from plastic and glass surfaces. It effectively removes high levels of RNase contamination.

Before Use

Shake or heat at 37℃ to bring the precipitate back to solution.

Cleaning work Surface

Apply RNZO directly to surface to be cleaned, wipe thoroughly with paper towel, rinse with water and then dry with clean paper towel.

Ordering Information

Category	Product Name	Cat No.	Size
RNA Care	RNZO	RN983048	25ml

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 260nm and 280nm 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 280nm. 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 20µl.
- Assemble the gel in the tank.
- Use TAE 1x buffer (%5 formaldehyde) as the tank buffer.

RNA sample preparation

- Add 10µg of purified RNA to 6µl of ROJE sample buffer (in RNA Loading Set, order by cat No LD983007) and incubate at 70 °C for 3 min.
- Add 3µl 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.

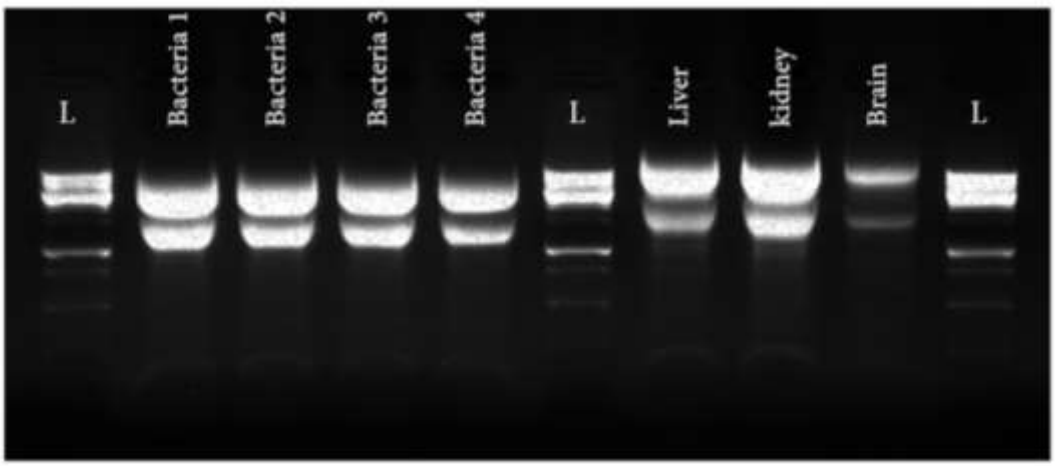

Figure 1. Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10 µg RNA was pretreated by ROJE Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

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

MiRJia 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 30 min.
- Centrifuge for 10 min at 14000 rpm in a benchtop centrifuge. Discard the supernatant.
- Wash the pellet with 100µl 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:

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

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 10µl 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

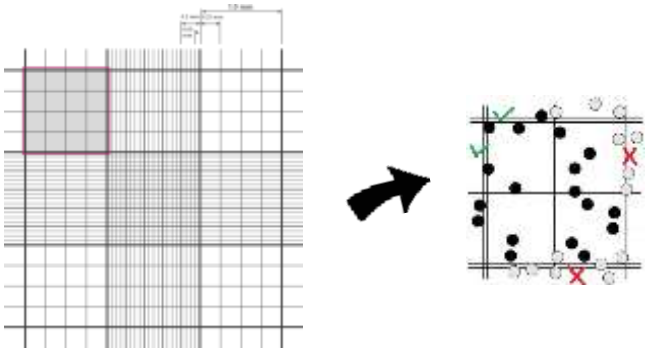

Figure2. Cell counting with hemocytometer

Appendix 8: Preparation of Phosphate Buffered Saline (PBS)

Dissolve the components in about 800mL dH2O. Adjust the pH to 7.4 with HCl. Adjust the volume to one liter. Sterilize by autoclaving. Store at room temperature.

Table 7. PBS preparation

Concentration	Component	G/litter
137mM	NaCl	8g
2.7mM	KCl	0.2g
10mM	Na2HPO4	1.42g
1.8mM	KH2PO4	0.25g

Factory address

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

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