Gene to Protein Pvt. Ltd.

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G2P-ZOL™ (Equivalent to TRIzol)

Cat # ☐**RNA001,** ☐**RNA001-S**
Pack Size: ☐**100 mL,** ☐ **8mL**
Storage: 4°C

Introduction

G2P-ZOL™ is a single-step reagent developed for the isolation of total RNA from various biological samples, including tissues and cells. Through a liquid phase separation process, it facilitates the separation of RNA from other cellular constituents such as DNA, proteins, polysaccharides, and additional biomolecules. This separation allows for the subsequent isolation of RNA from the supernatant using alcohol or isopropanol precipitation. The end product is high-quality RNA, characterized by distinct 28S (23S), 18S (16S), and 5.5S (5S) rRNA bands, with minimal to no contamination from DNA and proteins. G2P-ZOL™ offers the capacity to isolate RNA from up to 100 mg of tissue or 1 x 10^7 cells per milliliter, providing a robust and efficient solution for RNA-related research and diagnostic needs.

Precautions

- Personal Protective Equipment: Always wear appropriate personal protective equipment, such as gloves and a lab coat, when handling kit components.
- Handling of G2P-ZOL™ Reagent: Avoid direct skin contact with G2P-ZOL™ reagent, as it is a corrosive and irritant agent. Handle with care and follow safety guidelines.
- Nuclease-Free Containers: Utilize only nuclease-free tubes and pipette tips to prevent any potential contamination of the RNA samples.
- Sample Volume: Do not exceed the recommended maximum volume for the blood sample, as it may affect the efficiency and accuracy of the isolation process.

Procedure

1. Collect tissue samples and store them at -80°C if not using immediately.
2. When ready to process, transfer the tissue to a suitable tube containing G2P-ZOL™ reagent.
   The tube size for RNA isolation using G2P-ZOL™ will depend on the amount and type of sample being used. Here are some general guidelines:
   - Small samples (e.g., cultured cells or small tissue samples up to 50 mg): Use 1.5 mL microcentrifuge tubes
   - Medium samples (e.g., larger tissue samples up to 100 mg): Use 2 mL screw-cap tubes
   - Large samples (e.g., whole organs or larger tissues): Use 15 mL or 50 mL conical tubes, and scale up the volume of G2P-ZOL™ accordingly

Note: It is important to choose a tube size that allows enough room for the homogenizer probe or pestle to move freely and effectively homogenize the sample without causing excessive shear forces that could degrade the RNA.

3. Homogenize the tissue in G2P-ZOL™ using a homogenizer until the sample is completely disrupted
4. Incubate the homogenate at room temperature for 5-10 minutes to allow complete dissociation of nucleoprotein complexes
5. Add 200 µL chloroform to the homogenate and mix thoroughly by shaking or vortexing
6. Let the tube stand in a vertical position for 2–3 min.
7. Centrifuge the mixture at high speed (12,000-15,000 x g) for 15 minutes at 4°C
8. After centrifugation, the mixture will separate into three phases: a red organic phase (containing proteins), an interphase (containing DNA), and a colorless upper aqueous phase (containing RNA)
9. Carefully transfer the aqueous phase to a new tube using a pipette, being careful not to disturb the interphase
10. Add an equal volume of 100% isopropanol to the aqueous phase and mix well
11. Incubate at -20°C for 15 minutes
12. Centrifuge at 12,000 × g for 30 min at 4°C. Discard the supernatant
13. Wash precipitated RNA by adding 500 µL of 70% ethanol and mix by inverting the tube several times
14. Centrifuge at 12,000 x g for 5 min at 4°C; Carefully discard the supernatant without losing the pellet
15. Centrifuge the tube for a few seconds to bring any remaining liquid to the bottom of the tube which is then removed using a P100 micropipette
16. Air-dry the RNA pellet for 5 min to allow ethanol to evaporate. Do not over dry as it would decrease the solubility affecting final RNA concentration
17. Add 50 µL of TE buffer and store at -80°C for further use.

Approx Yield

It should have a A260/A280 ratio of 1.7- 2.1.
Typical RNA yields from tissues (mg RNA/mg tissue): liver, 6-8 mg; kidney, spleen, 3-4 mg; skeletal muscle, brain, lung, 0.5-1.5 mg; placenta, 1-3 mg.

Result

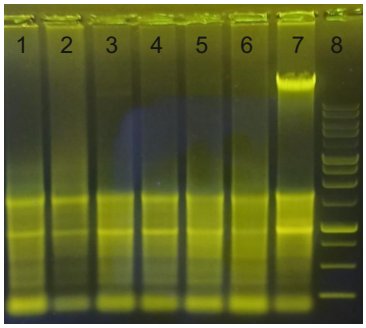

Figure: Total RNA isolation from Mouse brain using lanes: 1-6 G2P-ZOL™ lane 7 MRC TRI Reagent. 5 ul of Total RNA extracted was loaded in 1% denaturing gel. (Note 3 clear bands of 28S, 18S and 5.5S rRNA) Lane 8: 1 kb ladder

Troubleshoot

- **Cells:** If you are using cells, ensure that you are working with healthy and confluent cells. Over-confluent or stressed cells may have reduced RNA quality and yield.
- **Tissues:** When working with tissues, it is important to homogenize the sample thoroughly to maximize yield. If you are working with a particularly tough tissue, you may need to increase the amount of G2P-ZOL™ you use.
- **Blood:** For blood samples, ensure that you add the G2P-ZOL™ directly to the whole blood and mix it thoroughly. It is recommended to process blood samples within 2 hours of collection to avoid RNA degradation.
- **Bacteria:** When isolating RNA from bacteria, it is important to use a high-quality G2P-ZOL™ reagent and to ensure that the bacterial culture is in log-phase growth. High-density bacterial cultures may have lower RNA yield.
- **Fungi:** When working with fungi, it is important to use fresh and healthy cultures. Ensure that the fungal culture is thoroughly homogenized before adding G2P-ZOL™ to maximize yield.
- **Plant tissues:** Plant tissues are often high in polysaccharides, which can interfere with RNA isolation. To avoid this, it is recommended to grind the plant tissue in liquid nitrogen before adding G2P-ZOL™.
- **RNA degradation:** If you are experiencing low RNA yield or poor RNA quality, it is possible that the RNA is degrading during the isolation process. To prevent RNA degradation, work quickly and keep samples on ice when possible. It is also important to store RNA at -80°C immediately after isolation.
- **Contaminants:** Contaminants such as DNA and proteins can interfere with RNA isolation. To avoid this, ensure that you follow the instructions carefully and remove any visible contaminants during the isolation process. If the DNA contamination still there please treat it with DNase.
- **Equipment:** Ensure that all equipment used during RNA isolation, including centrifuges and pipettes, are properly calibrated and maintained. contamination free.
- If you are experiencing issues with RNA isolation from a specific source, it is recommended to consult the literature for the recommended protocol and any specific troubleshooting guidelines.

Note: This protocol is provided as a general guideline. Optimization may be necessary based on the specific sample type, source, or downstream applications. Careful consideration should be given to the sample's nature and the intended use of the isolated RNA to ensure successful isolation.