

Frequently Asked Questions

TIARIZOL™ REAGENT

1. What is TIARIZOL™ REAGENT?

TIARIZOL™ REAGENT is a ready to use solution designed to extract RNA, DNA, and proteins in three separate phases from the same sample. It is a monophasic solution composed of phenol and guanidinium thiocyanate.

2. How does TIARIZOL™ REAGENT work?

After sample homogenization, TIARIZOL™ REAGENT preserves RNA integrity while simultaneously disrupting cells and dissolving their components. Following the addition of chloroform and centrifugation, the mixture separates into two distinct phases: an aqueous phase and an organic phase. RNA is confined exclusively to the aqueous phase. The RNA is then isolated by transferring the aqueous phase and precipitating it with isopropanol. After the aqueous phase is removed, DNA and proteins can be extracted sequentially from the sample. DNA is obtained from the interphase by ethanolic precipitation, while proteins are recovered from the organic phase using isopropanol precipitation.

3. Can TIARIZOL™ REAGENT be used to isolate DNA?

Yes. DNA can be isolated from the interphase. Precipitation with ethanol followed by specific washes, yields high-purity DNA.

4. Can TIARIZOL™ REAGENT be used to purify proteins?

Yes. Proteins can be isolated from the organic phase after RNA is isolated from the aqueous phase and DNA from the interphase. However, proteins are denatured during the process, which limits their use in functional studies but makes them suitable for structural or analytical studies like SDS-PAGE, Western blotting and mass spectrometry.

5. What type of samples can TIARIZOL™ REAGENT be used on?

TIARIZOL™ REAGENT works with a wide variety of samples, including human, animal, plant, and bacterial cells cultured cells or tissues. It is suitable for small quantities (50–100 mg of tissue or 5×10^6 cells) and large quantities (≥ 1 g of tissue or 10^7 cells).

6. How long does the TRIZOL® Reagent RNA isolation process take?

The entire RNA isolation procedure can be completed in about one hour, making it suitable for processing a large number of samples simultaneously.

7. What downstream applications are compatible with RNA isolated using TIARIZOL® REAGENT?

Isolated RNA is suitable for Northern blot analysis, dot blot hybridization, RT-PCR, RNase protection assays, in vitro translation, molecular cloning, and poly(A)⁺ selection. For PCR applications, treat the RNA with DNase I, RNase free (TBZ0319) to remove residual DNA.

8. How do I ensure RNA integrity during the isolation process?

To protect RNA, follow these precautions:

- Always wear disposable gloves.
- Use RNase-free plasticware and equipment.
- Work in a clean environment.
- Avoid washing cells prior to homogenization to minimize RNA degradation.

9. How should RNA isolated with TIARIZOL™ REAGENT be stored?

RNA can be stored in RNase-free water at -70°C for long-term storage. Alternatively, it can be stored in 75% ethanol at 2–8°C for up to one week or at -20°C for longer durations.

10. What should you do if aqueous colorless layer after TIARIZOL™ REAGENT/ chloroform appear at the bottom phase?

RNA extraction protocol using TIARIZOL™ REAGENT indicates that you must take top colorless phase after addition of TIARIZOL™ REAGENT and chloroform. Sometimes it happens that aqueous colorless layer is at bottom position. Although there are quite a few reasons, inverted layers are usually due to use of larger amount of samples or/and excess salts in the samples. Solution is easy: add more TIARIZOL™ REAGENT volume or add 1/5 of Molecular Biology Grade Water (TBB0298-0301). Vortex the tubes and centrifuge.