

Product Application

Using the ReliaPrep™ RNA Cell Miniprep System to Isolate Bacterial and Mammalian RNA From Cell Lysates in TRIzol® Reagent.

Isolate high quality, amplifiable RNA from bacterial infected cells collected in phenol and guanidine isothiocyanate based products such as TRIzol® Reagent using ReliaPrep™. No need for phase separation or additional organic solvents.

Kit: Reliaprep[™] RNA Cell Miniprep System

Analyses: GoTag® RT-qPCR, QuantiFluor® quantitation

Sample Type(s): Cells infected with bacteria

Input: up to 500μl of TRIzol® or other phenol and guanidine isothiocynate based lysate.

Materials Required:

Reliaprep™ RNA Cell Miniprep System (Cat. #Z6011)

Protocol:

- 1. Add TRIzol® to cells as indicated in the TRIzol® Reagent technical manual up to the point where phase separation would be performed.
- 2. Add 35ul of isopropanol per 100ul of lysate.
- 3. Mix by vortexing for 5 seconds.
- 4. Transfer up to 500μ l of lysate to a ReliaPrepTM Minicolumn and centrifuge at 12,000-14,000 x q for 30 seconds at room temperature.
- 5. Continue with ReliaPrep™ protocol as described in TM starting with step 8.

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

For further information, please contact

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Results:

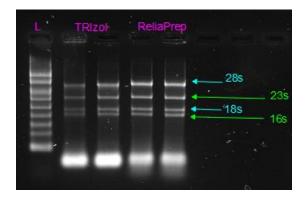


Figure 1: RNA isolated from a TRIzol® Reagent lysate containing 1.4E06 CFUs of E. coli and 2E05 RAW264.7 cells using the TRIzol® recommended protocol (TRIzol®) or the ReliaPrep™ RNA Cell Miniprep System (Reliaprep™) was run on a 1% agarose gel to determine quality.

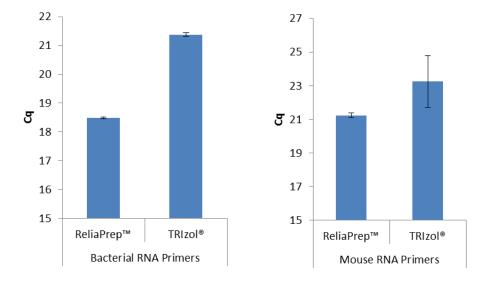


Figure 2: RNA was isolated from a TRIzol® Reagent RAW264.7 cell lysate spiked with E.Coli. Cq values are shown from RT-qPCR with Bacterial (Left) or Mouse (Right) specific primers using the GoTaq® One-Step RT-qPCR system.