

Using the ReliaPrep™ RNA Cell Miniprep System to Isolate Viral RNA From Cell Culture Media in TRIzol® LS Reagent.

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

Kit: Reliaprep™ RNA Cell Miniprep System

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation

Sample Type(s): Cell culture media containing virus

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

Materials Required:

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

Protocol:

1. Add TRIzol® to cell media 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 500µl of lysate to a ReliaPrep™ Minicolumn and centrifuge at 12,000-14,000 x g 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 techserv@promega.com

Results:

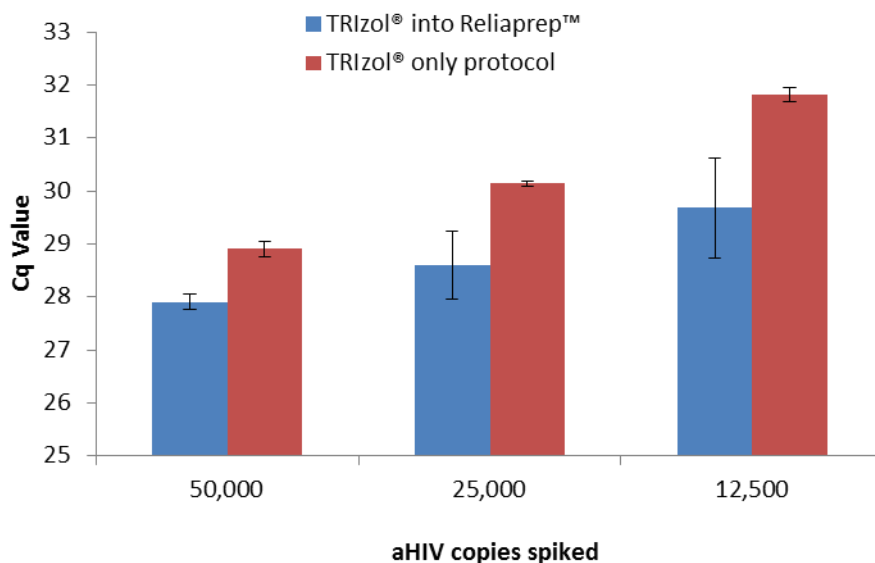


Figure 1: Cq values from RT-qPCR with RNA isolated from TRIZOL® LS treated cell culture media with the Reliaprep™ RNA Cell Miniprep System or by TRIZOL®. 50,000-12,500 copies of Armored® RNA HIV subtype b (Asuragen, Cat. #4200), viral RNA encapsulated in protein, was spiked into DMEM with 10% FBS and treated with TRIZOL® LS Reagent. Following TRIZOL® addition, samples were purified by TRIZOL® as described in the technical manual (TRIZOL® only protocol) or with the Reliaprep™ RNA Cell Miniprep System (TRIZOL® into Reliaprep™). RT-qPCR was run using 5µl of isolated RNA in the GoTaq® Probe 1-Step RT-qPCR System.