INVENTIA LIFE SCIENCE

RESEARCHER-SUBMITTED RASTRUM PROTOCOL

TRIzol Extraction of RNA from RASTRUM™ 3D Cell Models

Submitted by Esther Rodman, PhD, and Michael Emch, PhD,
Laboratory of Prof. John Hawse, PhD, Mayo Clinic

Introduction

RNA isolation is a critical step in analyzing gene expression in 3D cell models. This protocol outlines a streamlined method for isolating RNA from RASTRUM 3D cell models using TRIzol, ensuring reliable extraction while maintaining sample integrity. The protocol includes handling precautions and considerations for working with TRIzol in 3D cell cultures.

Handling of reagents

TRIzol is a monophasic solution of phenol and other components. All steps should be performed in a chemical fume hood.

Equipment and reagents required

- 1 x Dulbecco's Phosphate-Buffered Saline (DPBS) solution (ThermoFisher, 14190144 or similar)
- TRIzol (ThermoFisher, 15596026 or similar)

Protocol

1. Aspirate and discard media from wells by tilting plate at an angle to maximise fluid removal.

   Caution: Ensure pipette tip does not pierce the cell model or inert base.

2. Add 200 µL of DPBS and aspirate to wash models. Repeat for a total of 2 washes.

3. Add 50 µL of TRIzol directly to the well—this volume is sufficient to cover the surface of a well in a 96-well plate (the same volume can be used for both the Imaging Model and Large Plug Model, but should be adjusted for different plate sizes). Pipette the TRIzol up and down >10 times shortly after adding.

   Note: TRIzol can start to lift the inert base, but this will not impact the downstream protocol for RNA isolation. If disturbed, inert base debris can form a white precipitate with both TRIzol directly in the well or during later centrifugation steps. During the phenol-chloroform extraction, any white precipitate will likely accumulate in the middle interphase layer. Continue with the transfer of the upper aqueous phase as normal. The precipitate should not interfere with extraction.

4. Use in preferred downstream protocol for RNA isolation (e.g. Qiagen RNAeasy kit or RNA isolation protocol using TRIzol), and combine wells as needed.

Example yields

Cell line	RNA concentration (ng/µL)
HGSOC-1	185.15
HGSOC-2	388.70
HGSOC-3	292.75

Table 1. Typical RNA yields following TRIzol isolation and pooling of 8 wells per cell line from the Large Plug Model (5–10 million cells/mL) after 10 days of growth.

20-22 William Street, Alexandria, NSW, 2015 Australia

info@inventia.life | www.inventia.life