

STANDARD OPERATING PROCEDURE

SOP # 2	Version 1.1	Prepared By: Dr Joyanta Modak
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	Revise by: 08/08/2017	

Laboratory of Viral Evolution, Department of Microbiology, Monash University

SOP 2: Virus RNA isolation using TRIzol

1. INTRODUCTION

This SOP describes the use of TRIzol to isolate total RNA from sample.

2. SCOPE

This procedure serves as a guide to laboratory personnel engaged in viral RNA purification using the TRIzol.

3. SAFETY

All lab workers must:

- Wear appropriate personal protective equipment (PPE) (Nitrile gloves, lab coat, closed-toed shoes, and safety glasses when specified by risk assessment) when carrying out the procedure.
- If procedure is performed on reassortant viruses, please wear an N95 mask for additional personal protection.
- **USE TRIZOL IN FUMEHOOD** → always discard any Trizol/phenol/chloroform waste in cytotoxic waste bin under fume hood.
- **USE RNaseZap** spray to remove RNase contamination from work surface/ equipment/ solutions.
- Read and understand the recommendations in this SOP to ensure uniformity in practice.
- New staff or students should seek supervision from a senior lab co-worker during first attempt of the procedure.

4. PROCEDURES

4.1 Precaution

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use cold TRIzol™ LS Reagent if the starting material contains high levels of RNase, such as spleen or pancreas samples.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.

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- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials.
- Always use proper microbiological aseptic techniques when working with RNA.
- Use RNaseZap™ RNase Decontamination Solution to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipettes used during purification.

4.2 Materials Needed

- Virus-infected cell-culture supernatant
- TRIzol LS reagent, Invitrogen, Cat # 10296010
- RNAZap (Cat. no. AM9780)
- Ethanol 75%
- Isopropanol
- Chloroform
- 1.5 mL micro-centrifuge tubes (Axygen Cat# MCT-150-C)
- ART 200 and ART 1000XL aerosol barrier pipette tips (Molecular Bio-Products Cat# 2069-05-HR and 2179-05-HR)
- P100 and P1000 single-channel pipettes (Eppendorf Cat # 3111000.149 and 3111000.165)
- Micro-centrifuge capable of reaching $12,000 \times g$ and 4°C
- Water bath or heat block at $50 - 60^{\circ}\text{C}$
- Freezer -20°C
- Freezer -80°C

4.3 Sample Preparation

- Always maintain a ratio of 3:1 between the volume of TRIzol™ LS Reagent and the sample.
- To facilitate isolation of RNA from small quantities of samples ($<10^6$ cells or <10 mg of tissue) or for sample volumes <0.25 mL, adjust the sample volume to 0.25 mL with RNase-free water.

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4.4 Lyse samples and separate phases

- Add 0.75 mL of TRIzol reagent per 0.25 mL of sample volume.
- Homogenize the sample by pipetting up and down several times.
- (Optional) If samples have a high fat content, centrifuge the lysate for 5 minutes at $12,000 \times g$ at $4-10^{\circ}\text{C}$, then transfer the clear supernatant to a new tube.
- Incubate for 5 minutes at room temperature to permit complete dissociation of the nucleoproteins complex.
- Add 0.2 mL of chloroform per 0.75 mL of TRIzol reagent used for lysis, then securely cap the tube.
- Incubate for 2–3 minutes at room temperature.
- Centrifuge the sample for 15 minutes at $12,000 \times g$ at 4°C .
- The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.
- Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.
IMPORTANT: Avoid transferring any of the interphase or organic layer into the tube when removing the aqueous phase.
- Proceed directly to Isolate RNA steps.

4.5 Isolate RNA

1. Precipitate the RNA
 - a. Add 0.5 mL of isopropanol to the aqueous phase, per 0.75 mL of TRIzolTM LS Reagent used for lysis.
 - b. Incubate for 10 minutes.
 - c. Centrifuge for 10 minutes at $12,000 \times g$ at 4°C .
Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
 - d. Discard the supernatant with a micropipette.
2. Wash the RNA
 - a. Resuspend the pellet in 1 mL of 75% ethanol per 0.75 mL of TRIzolTM LS Reagent used for lysis.
Note: The RNA can be stored in 75% ethanol for at least 1 year at -20°C , or at least 1 week at 4°C .

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- b. Vortex the sample briefly, then centrifuge for 5 minutes at $7500 \times g$ at 4°C .
 - c. Discard the supernatant with a micropipettor.
 - d. Vacuum or air dry the RNA pellet for 5–10 minutes.
IMPORTANT! Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet dry, to ensure total solubilization of the RNA. Partially dissolved RNA samples have an $A_{230/280}$ ratio <1.6 .
3. Solubilize the RNA
 - a. Resuspend the pellet in 20–50 μL of RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution by pipetting up and down.
IMPORTANT! Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.
 - b. Incubate in a water bath or heat block set at $55\text{--}60^{\circ}\text{C}$ for 10–15 minutes.
 - c. Proceed to downstream applications, or store the RNA at -70°C .
4. Determine the RNA yield
 - a. RNA samples can be quantified by absorbance without prior dilution using the NanoDropTM Spectrophotometer.
 - b. Calculated A_{260}/A_{280} ratio of ~ 2 is considered pure.

5. REFERENCES

1. Invitrogen TRIzol reagent Protocol.
2. Chomczynski, P., and Sacchi, N. 1987 Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. Anal. Biochem. 162, 156-159.