OSUCCC LTB Laboratories Procedure Preparation of RNA lysate from mononuclear cell fraction[1]			Effective: 10/01/1997
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1.0 PRINCIPLE

TRIZOL® Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues manufactured by Invitrogen Corporation. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate (GITC), is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi.[1] During sample homogenization or lysis, TRIZOL® Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components.

RNA STAT-60™ is a newer reagent that may also be used in the single-step RNA isolation method and is manufactured by Tel-test B. It is a complete and ready to use reagent for isolation of total RNA from tissues and cells of human, animal, plant, yeast, bacterial, and viral origin. The composition of RNA STAT-60™ (patent pending) includes phenol and Guanidinium thiocyanate in a mono phase solution. A biological sample is homogenized in the RNA STAT-60™ using a glass-Teflon or Polytron homogenizer.

[1] T:\HCG\Caligiuri lab\Procurement\Lab Manual\Protocols\ALLIANCE-OSU LTB

2.0 SPECIMEN

Mononuclear cell fractions are prepared by ficoll and cells are suspended in sterile, isotonic buffer solution such as Dulbecco's PBS (Ca/Mg free). Cells are obtained from bone marrow or peripheral blood collected with an anticoagulant such as heparin or EDTA. ALLIANCE or OSU treatment protocols specify collection methods for samples requiring RNA isolation. Samples for all ALLIANCE studies should be collected into the appropriate collection tube indicated in the study protocol. Any problems or comments regarding sample collection, shipment and receipt will be noted by the technician on the sample procurement form. For non-ALLIANCE study samples, the Procurement lab will be notified that a patient sample is waiting in the 2nd floor Oncology lab, James Cancer Hospital for processing.

3.0 MATERIALS AND SUPPLIES

TRIZOL® solution or RNA STAT-60™solution (specified by Study Chair)
Sterile pipets (5ml)
Sterile 4x4 gauze
70% isopropyl alcohol
Sterile RNase-free eppendorf tubes (.650 or 1ml)
Pipet-aid
Sterile pipet tips (200µl and 1000 µl)
Micropipetters (20-200µl and 1000 µl)

4.0 EQUIPMENT

Biosafety cabinet
Microcentrifuge

5.0 QUALITY CONTROL AND SAFETY

RNA STAT-60™ and TRIZOL® reagents are both sensitive to temperature and light. During use, keep reagent container covered in foil and on wet ice. Both RNA STAT-60™ and TRIZOL® may be used the same way for the same application, but they are two different products. **It is important to refer to each by its own name when recording sample preparation method in written log sheet.** For the purpose of this protocol, both reagents will be referred to as the lysis buffer.

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. Always wear disposable gloves as skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination. Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment.

It is recommended that specimen collection be carried out in accordance with NCCLS document M29T2. No known test sample can offer complete assurance that human blood samples will not transmit infection. Therefore, all derivatives are potentially infectious. Always spray alcohol on the caps before opening solutions. The alcohol can be dried off using gauze. If you have been out of the hood for a while and are wearing the same pair of gloves, use a new pair of gloves. Remember not to touch the sides (inside or outside) of any bottles with your pipette – if you do, dispose of the pipette and start again.

6.0 PROCEDURE

6.0.1. RNA lysate preparation with TRIZOL® or RNA STAT-60™ requires a known population of cells to be resuspended in the reagent. Once a cell count is performed on cells suspended in a PBS solution, calculate volume needed for RNA lysate preparation.

6.0.2. Calculation: Divide the desired number of cells (*A*) by the number of cells per mL of the suspension (*S*) to get the volume of suspension to be aliquoted in mL (*V*). (Make sure that your units match.) $\frac{A}{S} = V$

6.0.3. Aliquot the correct volume of cell suspension into a sterile, DNase, RNase free eppendorf tube. Tube should be labeled with sample accession number and date.

6.0.4. Spin cell suspension in microcentrifuge at 2500rpm for 5 minutes.

6.0.5. During spin, place the lysis buffer being used on ice, put ice bucket in the hood.

6.0.6. After cell suspension has been pelleted, gently remove supernatant with a pipet. Do not pour off supernatant.

6.0.7. Gently but thoroughly resuspend cell pellet as follows: lysis buffer should be on ice

Cell number = 4 million to 10 million cells – resuspend pellet in 1 ml RNA lysate reagent
Cell number = less than 4 million cells – resuspend pellet in 500 µl RNA lysate reagent

6.0.8. When resuspending, gently pipet pellets no more than 4 times. Immediately cap eppendorf tube and place samples on ice. Make sure that tubes stay upright.

6.0.9. When all samples are complete transfer eppendorf tubes to the appropriate box (Labeled with the matching study number) in the -80C freezer, Room 334 MRF.

6.1 SPECIMEN PREPARATION NOTES[2]

ALLIANCE 9621 molecular analysis sample: Prepare one RNA lysate at 5 million cells. If cell number is limited, use total cell count, unless it is below 1million. For 9665: When ample cells are available, prepare 2 to 5 RNA stat preps at 5 million cells per prep.

ALLIANCE 19808 molecular analysis sample: Prepare one RNA lysate at 5 million cells. If cell number is limited, use total cell count, unless it is below 1million. For 9665: When ample cells are available, prepare 2 to 5 RNA stat preps at 5 million cells per prep.

ALLIANCE 29801: When ample cells are available, prepare 2 RNA lysate preps at 10 million cells per prep. If cell number is limited split the sample; prepare half for viable cells and half for RNA lysate in two tubes. The smallest split should be 2 million cells in each RNA prep; if there is too small a cell count for this, bank viable samples only.

ALLIANCE 10503: Prepare one RNA lysate at 5 million cells. If cell number is limited, bank viable cells only.

7.0 LIMITATIONS OF THE PROECURE

Low yield of RNA from subsequent isolation may be directly related to incomplete homogenization of sample with lysate buffer. Samples stored at 4°C or -20°C will yield RNA which is degraded so it is critical that lysates are stored at -80°C.

8.0 REFERENCES

[2] http://www.Alliance.org/Private/COOP_Groups/ALLIANCE/studies/studies.php

1. Chomczynski P. and Sacchi N. (1987) *Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction*. Anal. Biochem. 162, 156-189.