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| STANDARD OPERATING PROCEDURE (SOP) |

## STANDARD OPERATING PROCEDURE DETAILS

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| SOP Title: | Inhouse Method for Total RNA Extraction |
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## DECLARATIONS

I have read this document and approve its contents.

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| --- | --- | --- | --- | --- |
|  | Name | Team | Signature | Date |
| Written By: | Subash Kumar Rai | Long Read Sequencing |  | 28 Oct 2021 |
| Reviewed By: |  |  |  |  |
| Reviewed By: |  |  |  |  |
| Reviewed By: |  |  |  |  |
| Reviewed By: |  |  |  |  |
| Authorised By: | Brooke Purdue |  |  |  |

## DOCUMENT HISTORY

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#### PURPOSE AND APPLICATION

This SOP covers required equipment, materials, consumables, procedures, and anticipated results along with worked example with RNA quality. The method described herein is suitable for any total RNA studies including RNA sequencing such as RNA-seq (Illumina), Isoseq (PacBIO), full length cDNA and direct RNA-seq (ONT).

#### BRIEF SUMMARY OF METHOD

The method described in this SOP is modified from Acosta-Maspons, González-Lemes & Covarrubias (2019) and TRIzol User Guide (15596026, Life Technologies). The extraction part is adopted from Acosta-Maspons *et al.* (2019) and the final RNA precipitation steps are modified based on TRIzol method. This method obtains high quality total RNA from different sources such as plant roots, leaves, and microalgae.

#### DEFINITIONS AND ABBREVIATIONS

CTAB: Cetyltrimethylammonium Bromide

EDTA: Ethylenediaminetetraacetic Acid

SDS: Sodium Dodecyl Sulfate

NaCl: Sodium Chloride

RT: Room Temperature

#### OCCUPATIONAL HEALTH AND SAFETY

No OH&S risk is identified with this SOP.

#### CAUTIONS

User should work under the fume hood while working with β-mercaptoethanol, Chloroform: Isoamyl alcohol (24:1), and TRIzol.

#### PERSONNEL QUALIFICATIONS, TRAINING AND RESPONSIBILITIES

Training Requirements:

X Read and Understand Document X Training Required

#### EQUIPMENT AND MATERIALS

##### 

##### Equipment

* 1. Mortar and pestle
  2. Mini centrifuge
  3. Vortex
  4. Tabletop centrifuge at 4°C
  5. Heat block
  6. Esky/container for ice
  7. Flask Dewar or equivalent to transport LN2
  8. Qubit
  9. NanoDrop
  10. P1000 pipette
  11. P200 pipette
  12. P100 pipette
  13. P10 pipette

##### Materials and Consumables

* 1. UltraPure 1M Tris-HCI, pH 8.0 (15568025, Life Technologies Australia)
  2. CTAB (52365-50G, Sigma Aldrich)
  3. EDTA (0.5M), pH 8.0, Nuclease-free (AM9260G, Life Technologies Australia)
  4. Sodium Chloride (71580-500G, Sigma Aldrich)
  5. Chloroform: Isoamyl alcohol (24:1) (ACR327155000, Thermo Fisher Scientific Australia)
  6. SDS 20% solution, RNase free (AM9820, Life Technologies Australia)
  7. Ethanol (>98%, US015017, Thermo Fisher Scientific)
  8. Lithium Chloride solution, 8M (L7026-100ML, Sigma Aldrich)
  9. Isopropanol (>98%, W205702-1KG-K, Sigma Aldrich)
  10. β-mercaptoethanol (M6250-100mL, Sigma Aldrich)
  11. Spermidine trihydrochloride (S2501-5G, Sigma Aldrich)
  12. Distilled water Ultra-Pure Dnase/Rnase free Gibco 500ml
  13. Qubit RNA HS Assay Kit (Q32855, Life Technologies Australia)
  14. RNaseZap® Solution (AM9780, Life Technologies Australia)
  15. TURBO DNA-free™ Kit (AM1907, Life Technologies Australia) optional
  16. 15ml and 50ml Falcon tubes
  17. Eppendorf tubes 1.5ml and 2ml
  18. Liquid Nitrogen
  19. Dry Ice

#### PROCEDURES

Before sample processing, prepare following buffers and solutions:

a. RNA extraction [100mM Tris-HCl, 25mM EDTA, 2% CTAB (w/v), 2M (NaCl), 0.75 g/L Spermidine trihydrochloride, 4% β-mercaptoethanol]:

Add 5ml 1M Tris-HCl (pH = 8), 2.5ml of 0.5M EDTA (pH = 8), 0.0375g Spermidine trihydrochloride, 1g of CTAB powder and 5.844g of NaCl in a 50ml Falcon tube. Complete to 50ml with nuclease free water. Add 4% β-mercaptoethanol just before use.

b. Resuspension buffer SSTE[1M NaCl, 0.5% SDS (w/v), 10mM Tris-HCl (pH = 8.0), 1mM EDTA)]:

Add 0.25ml SDS (20%), 0.2ml of 1M Tris-HCl (pH = 8), 0.04ml of 0.5M EDTA (pH = 8) in 8ml nuclease free water and add 1.167g NaCl and adjust final volume to 10ml with nuclease free water. This will form cloudy solution but will become clear solution after incubating at 65°C for 5-10min.

1. Incubate 15ml of extraction buffer at 65°C for at least 30min. Add 4% β-mercaptoethanol just before use.

2. Clean the working bench, mortar-pestle, pipettes, and tube racks with RNAZap.

3. Chilled the mortar-pestle and grind fresh/snap frozen tissues/cells (e. g. 200-800mg for plant leaves/roots or cell pellets) in LN2 till finely ground (it may require topping up LN2 1 or 2 times).

4. Place a falcon tube (15ml) in dry ice to chill it and swirl ground fine powder with pestle and pour ground tissues/cells powder-LN2 into 15ml Falcon tube. Top-up LN2 in the mortar and try to pour all tissues powder-LN2 solution into the falcon tube. Cap the falcon tube's lid loosely so that LN2 evaporates out.

5. Add preheated (at 65°C) extraction buffer (do not forget to add 4% β-mercaptoethanol), followed by vigorous homogenization (vortex followed by P1000 pipetting) after which samples are kept on ice. Perform following step under fume hood. Perform the following steps under fume hood.

6. Add 1000µl of chloroform: isoamyl alcohol (24:1), mix well and centrifuge for 10min at 10000xg at 4°C.

7. Carefully transfer supernatant to a new 2ml tube (approximately 900µl).

8. Repeat steps 6 and 7; then add the appropriate amount of LiCl to 2M final concentration (from 8M LiCl starting solution add ½ v/v).

9. Leave precipitating overnight at 4°C (19h).

10. Centrifuge at 17000xg for 30min at 4°C.

11. Decant the supernatant and resuspend pellet in 100µl of preheated (65°C) SSTEby shaking. To avoid undesired precipitates, do not set samples on ice.

12. Add equal volume chloroform: isoamyl alcohol (24:1), mix well and centrifuge for 10min at 10000xg at 4°C, transfer supernatant to a new 1.5ml tube, and then keep on ice.

13. Add 1 volume of 2-propanol (v/v), mix gently, and incubate 10min at RT.

14. Centrifuge at 17000xg for 10min at 4°C, then pipette off the supernatant.

15. Wash pellet with 500µl ethanol 80%, dislodge the pellet by 2sec pulse vortexing and then centrifuge at 17000xg for 5min at 4°C. Repeat this step once.

16. Dry pellet 5 -10min on fume hood and resuspend in 50µl of nuclease free water and incubate for 10min at 65°C and put on the ice. Perform QC in NanoDrop and qubit.

17. Treat sample with TURBO DNA-free™ Kit according to manufacturer's instruction if DNA appears as contamination. This step requires if tissues amount is >200mg are used in RNA extraction.

18. Store the RNA at -80°C.

#### WORKED EXAMPLE

Table 1: Qubit and NanoDrop QC for total RNA extracted from different sources.



**A.** Chart, histogram

Description automatically generated **B.** Chart, histogram

Description automatically generated **C.** Chart, histogram

Description automatically generated with medium confidence

**Figure 1**. BioAnalyzer tracer showing the consistent good quality of total RNA extracted from different sources – roots (A), leaves (B), and microalgae (C). Distinct sharp peaks indicate good RNA integrity although the RIN values vary among the recourses having different RNA profiles.

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#### SOP VALIDATION DETAILS

The method described in this SOP has been tested in several samples from different resources and resulted consistent sample quality and quantity.

#### WASTE MANAGEMENT AND DISPOSAL

All the chemicals used in this SOP are very low amount. Collect TRIzol and Chloroform:Isoamyl alcohol waste in separate collection waste bottle and remaining waste in the clinical waste bin.

#### DATA RECORDS MANAGEMENT

All the data generated during QC should be recorded on lab book or digital lab book as soon as the experiment is completed.

#### REFERENCE DOCUMENTS

1. User Guide (MAN00001271) for TRIzol (15596026, Life Technologies), 29 Jan 2020.

2. Acosta-Maspons, A., González-Lemes, I.& Covarrubias A.A. (2019). Improved protocol for isolation of high-quality total RNA from different organs of Phaseolus vulgaris L. *Biotechniques*. 66(2). DOI: [10.2144/btn-2018-0129](https://doi.org/10.2144/btn-2018-0129).

#### QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA) SECTION

To get a good performance of this SOP, user should –

1. start with good quality starting materials

2. LiCl precipitation should not be longer than 19h, longer incubation results nonspecific precipitation

3. the better grinding of tissues or cells the better RNA yield