

09 Isolation of total RNA from adipose tissue with TRIzol UiO

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Transcriptomics

Keywords: Isolation, TRIzol, adipose tissue, RNA

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We used the NuGO Standard Operating Procedure (SOP) number 09 produced by the Institute for Nutrition Research, Faculty of Medicine, University of Oslo Details of the SOP are available via the web link:

<http://www.nugo.org/frames.asp?actionID=28250&action=loginFromPP>

Backgrounds

--to be edited--

Overview

TRIzol can be used to isolate total RNA from cells and tissues. TRIzol is a monophasic solution of phenol and guanidine isothiocyanate. Guanidinium isothiocyanate and chloride denature proteins and inhibit RNases. Denaturation of proteins ruptures the cell structure, and nuclear proteins will dissociate from the nucleic acids. Addition of chloroform provides a phenol-chloroform extraction of RNA. Centrifugation separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. RNA can be precipitated from the aqueous phase with isopropyl alcohol.

Materials

Amount	Name	Supplier	Catalogue Number	Further information
2ml	TRIzol	Invitrogen	15596-018	
	Liquid nitrogen			
	Ultra-turax			
0.2ml/ml TRIZOL	Chloroform			
0.5ml/ml TRIZOL	Isopropyl alcohol			
	Ethanol			75%
10µl	DEPC-H ₂ O			

Main Procedures

- Transfer tissue to tube containing TRIzol, homogenise and incubate
- Transfer TRIzol phase and add chloroform
- Centrifuge and add isopropyl alcohol
- Add DEPC-H₂O to RNA, which has formed a pellet, incubate and store at -70°C

Sub Procedures

1) Keep tissue in liquid nitrogen

Prior to homogenisation.

2) Transfer to tube containing TRIzol

Take tissue out of its wrapping and carefully, to avoid thawing, transfer to a tube containing 2ml TRIzol, and then homogenise using an ultra-turax.

3) Incubate at 30°C

-15 minutes

To permit complete dissociation of nucleoprotein complexes, incubate the homogenised samples for 15 min at 30°C.

4) Remove lipid layer

Remove the lipid layer on top of the sample and transfer the TRIzol-phase to a new tube.

5) Add chloroform - 5 minutes

Add Chloroform (0.2ml /ml TRIzol) and vigorously shake the samples for 15 sec at room temperature, followed by 3 min incubation at room temperature.

6) Centrifuge - 15 minutes

Centrifuge the samples at 15.000 rpm for 15 min at 4°C, and transfer the RNA to new tubes (the RNA is in the upper, aqueous phase).

7) Add isopropyl alcohol

-20 minutes

To precipitate the RNA add isopropyl alcohol (0.5ml/ml TRIzol). Mix the samples and incubate for 10 min at room temperature and centrifuge at 15.000 rpm for 10 min at 4°C.

8) Dissolve pellet in DEPC-H₂O (10 µl) and incubate - 20 minutes

After precipitation, the RNA is visible as a gel-like pellet on the bottom of the tube. Remove the supernatant and wash the pellet with 75% ethanol. Remove the pellet and leave to air-dry (not completely) for approximately 5 min. Re-dissolve in DEPC-H₂O (10 µl) and incubate at 55°C for 10 min.

9) Isolation complete

Store RNA at -70°C

Safety

Users must comply with COSHH and local safety regulations.