INRA - RENNES - Beaulieu **LPGP**		Code/Acronyme : A. Perquis Date création : 2021/07/27 Mise à jour : Version : 1
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Total RNA extraction from tissues with Trizol

This technic allows the extraction of total RNA from animal tissues or cells pellets

The extraction and purification of total RNA follow the protocol of Chomczynski *[Chomczynski P. and Sacchi N._1987: Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction.]* The organs are ground into the Trizol solution containing guanidium isothiocyanate (Rnases inhibitor), phenol (organic solvent capable of solubilizing proteins) and sodium acetate (allows the precipitation of the nucleic acids during the subsequent steps). After addition of choloroform, the samples are centrifuged in order to separate proteins (lower organic phase) and deoxyribonucleic acid (DNA) (interphase) from total RNA (upper aqueous phase). The aqueous phase is extracted and the total RNA is reconcentrated by conventional alcoholic precipitation.

REAGENTS AND CONCUMABLES

Trizol : TriReagent *(Euromedex, ref : TR118)* → storage at 4°C during 12 month

Chloroform *(Carlo Erba, ref : 438603)*

Ethanol *(Carlo Erba, ref : 4146012)*

Isopropanol *(Sigma, ref : 33539)*

CK14 ceramic beads 1,4 mm, tubes 0.5 ml *(ref P000933-LYSK0-A.0 OZYME)*

CK14 ceramic beads 1,4 mm, tubes 2.0 ml *(ref P000912-LYSK0-A.0 OZYME)*

CK14 ceramic beads 1,4 mm, tubes 7.0 ml *(ref P000940-LYSK0-A.0 OZYME)*

INFORMATIONS

Frozen Tissue Weight	*Volume of Trizol*	*Ceramic beads tubes*
100 mg	1 mL	2,0 mL
50 mg	500 µL	2,0 mL
25 mg	250 µL	0,5 mL

PROTOCOL

1. Take the samples out of the freezer and place them in liquid nitrogen until use.
2. Weight the tissue.
3. Place the tissue in a grind tube specific of the Precellys device.
4. Add Trizol TriReagent: 1mL for 100 mg of tissue
5. Grind tissue with the Precellys at room temperature:

Grind tubes	*Volum TriReagent*	*Program*
0.5 mL	< 0.250 mL	2 * 15 sec with break of 30 secs
2.0 mL	< 1.5 mL	2 * 20 sec with break of 30 secs
7.0 mL	< 5.0 mL	2 * 20 sec with break of 30 secs, twice

   a. Place the tubes in the Precellys

   b. Start the program according to the tissues and the volume

⇨ *No pieces should remain after grinding*

   c. Transfer into 1.5 mL, 2.0 mL or 5.0 mL eppendorf tubes depending on volume

6. Add chloroform: 200 µL for 1.0 mL of Trizol

   a. Mix quickly by hand during 15 secs

⇨ *Don't use the vortex*

   b. Incubation 5 - 10 mins at room temperature

   c. Centrifugation: 12 000 G / 4°C / 15 to 30 mins depending on the tissue

   d. Collect the colorless upper phase and transfer it in a new eppendorf tube

7. Add Isopropanol: 0.5 mL for 1.0 mL Trizol → *Allows to precipitate the RNA*

⇨ *Vortex quickly*

   a. Incubation : 2h / -20°C

   b. Centrifugation : 25 mins / 12 000 G / 4°C

   c. Remove the supernatant

8. Wash the pellet in 75% Ethanol: 1 mL for 1.0 mL Trizol

    a. Add 75% EtOH (-20°C) on the opposite wall of the pellet

    b. Centrifugation : 15 mins / 12 000G / 4°C

    c. Remove the supernatant gently

9. Resuspend the pellet with 75% Ethanol: 0.5 mL for 1.0 mL Trizol

    a. Add EtOH 75% against the reverse wall to the pellet

    b. Centrifugation : 10 mins / 12 000G / 4°C

    c. Remove the supernatant gently

    d. Quick centrifugation to remove all supernatant

    e. Let the pellet dry in the open air

10. Dissolve the pellet in Rnase- and DNase-free water.

11. After having done the quantification with Qubit or Nanodrop, store the RNA at -80°C.

BUFFER

75% Ethanol:

75 mL EtOH absolute

25 mL Water DEPC