1. Application field

Very rapid extraction method for small volume samples (too expensive for big samples volumes) ; usable for RNA preparation for Q-PCR, followed by **RNAeasy minielute (Qiagen)** column purification.

2. Diffusion list

BPMP

3. Referent person:

Philippe Nacry

4. Health and Safety :

TRizol Hazards :

Hazard pictograms

Signal word
Danger

hazard statements
H301 + H311 + H331 - Toxic if swallowed, in contact with skin or if inhaled
H314 - Causes severe skin burns and eye damage
H335 - May cause respiratory irritation
H341 - Suspected of causing genetic defects
H373 - May cause damage to organs through prolonged or repeated exposure
H412 - Harmful to aquatic life with long lasting effects

Precautionary Statements

Prevention
P201 - Obtain special instructions before use
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray
P264 - Wash hands thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P273 - Avoid release to the environment

Use items of personal protection: gloves, lab coat, eye protection (Liquid Nitrogen)
Manipulate TRIZOL under fume hood

	Rédacteur	Vérificateur	Approbateur
Nom : Visa :	Philippe Nacry		C Tournaire

5. Materials and chemicals

TRIzol or TRI REAGENT
Chloroform / isoamylic alcohol (24/1)
Glycogen (RNA carrier)
Ethanol 75 % in DNase free water (stored at -20 °C until use)
Isopropanol
ddH_2O (DNase/RNAse free H2O milliQ autoclaved)
Steel Beads for grinding mill
Liquid Nitrogen

6. Operating mode

Before using the beads, wash steel beads respectively with :
1. Absolute Ethanol
2.Water with detergent
3. 3X in milliQ water
4. 2X Absolute Ethanol
Dry and sterilize in oven.

- Centrifugations are performed in Eppendorf 5 810 R, with a rotor F-45-30-11. rotor, at 14 000 rpm et 12 000 rpm corresponding respectively to 20 817 g et 15 294 g.

- Fresh tissues (roots or leaves, from approximatively 300 plants cultivated for 8 days on verticals dishes petri dishes (few dozen mg FW) are sampled in 2 ml eppendorfs tubes and frozen in liquid nitrogen. Samples can be stored -80 °C before RNA extraction.

Dry the root sample on paper before freezing excess of water will reduce grinding efficiency and RNA yield

1. Grinding tissues :

Add one steel bead per tube. Place the tubes in the mill holder (pre refrigerated at -50 °C. Do not place the tube holder in liquid nitrogen), equilibrate the tubes in holders to avoid unbalance when shaking, and put 1 paper sheet (Whatmann paper) under each holder lid to prevent shocks and avoid tube lead breakage).

Grind 1 min at 25 shacking.sec^{-1}.

grind again 1 min à 25 shacking.sec^{-1}.

Samples should be finely ground. If necessary, refrigerate holders and tubes 10 min à -80 °C and grind again.

Return quickly samples to liquid nitrogen

Add 800 µL **TRIzol (under fume hood)**. Shake vigorously few seconds

Caution : check carefully tubes. If any breakage or leakage is detected, transfer the solution to new tubes.)

For tissues > 100 mg FW, add 8 µl TRIzol / mgFW. For following steps, adjust consequently volumes of solution.

1. **Incubate** 5 min at room temperature under constant shaking.
2. Add 160 µL de **chloroform / isoamylic alcohol** (24/1) **(under fume hood)**, **vortex** 15 sec and **incubate** 3 min at room temperature under constant shaking
3. **Centrifuge** 10 min at 14 000 rpm. At 4 °C.

4 Label a **new 1.5 ml tube series**. Add in these tubes 35 µg **glycogen** (1 µL of a 35 $\mu g.\mu L^{-1}$solution). In original protocol : 10 µg glycogène, instead of 35 µg).

Rq : glycogen helps RNA precipitation. It is not necessary if samples FW > 50 mg.

2. **Transfer aqueous phase** (upper phase, approximately 450 µL, collected under fume hood) in labelled new tubes. Discard tubes with balls phénol –chloroform Trash).
3. Add 400 µL **isopropanol**, **mix by inverting tubes** and **incubate** 10 min at room temperature.

Rq : incubation may be longer, at 4 °C (lunch time,…). For small samples FW > 20 mg place the tubes at least 30 min at -20°C to increase precipitation efficiency.

4. **Centrifuge** 15 min at 14 000 rpm., 4 °C.
5. **Discard** the supernatant.
6. **Wash the Pellet:** add 800 µL **75 %** Ethanol stored at -20°C. Mix by inverting.
7. **Centrifuge** 10 min à 12 000 rpm, 4 °C.
8. **Discard** the invert tubes on a paper towel to eliminate drops. **Dry** the pellet under vacuum 5 to 10 minutes (do not over-dry the pellet otherwise it may be difficult to resuspend).

Rq : From this step, **use filter tips**.

1. **Resuspend the pellet** in 87,5 µL **H_2O DEPC (or autoclaved milliQ water)**. Place tubes on ice. Resuspend the pellets with a pipet. If necessary, incubate 5 min at 60°C to help dissolution.