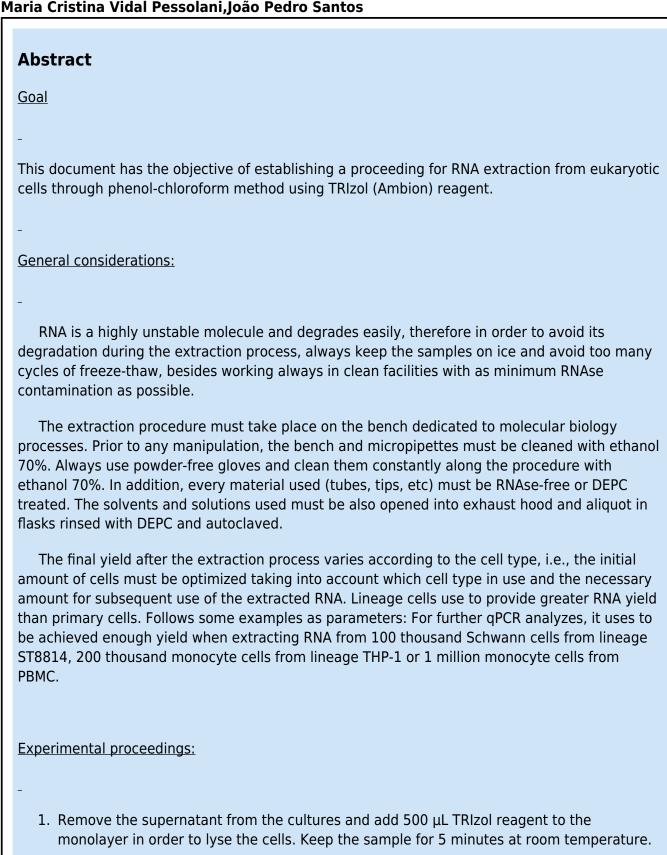




## Proceedings for RNA extraction from cell cultures using phenol-chloroform method with TRIzol ® (Ambion) reagent



- 2. Homogenize with micropipette 3 times, washing the well, and scrape the bottom of the plate with the tip to aid the lysis of the remaining cells. Recover all the TRIzol volume and transfer to a 1.5 mL microtube. Keep the samples on ice whilst the other samples are processed, until proceed to the next step.
- 3. Leave the cell lysates in TRIzol for 2-3 minutes at room temperature.
- 4. Add 100  $\mu$ L Chloroform: Isoamyl Alcohol (24:1), homogenize vigorously with the hand until it develops a milky aspect and incubate for 5 minutes at room temperature.
- 5. Centrifuge at 12,000 x g for 4 minutes at 4  $^{\circ}$
- 6. Transfer the aqueous phase (upper) to a new 1.5 mL microtube, minding not to collect the organic phase (middle) containing DNA and proteins.
- 7. Add 250  $\mu$ L isopropanol to the aqueous phase previously collected, homogenize gently the microtube twice and leave the RNA precipitating at -70  $^{\circ}$ C on the freezer for at least 30 minutes.
- 8. After the incubation, add 1  $\mu L$  GlycoBlue (Ambion) and centrifuge at 14,000 x g for 30 minutes at 4  $^{\circ}$
- 9. Observe the formation of a blue pellet on the bottom of the microtube and remove the supernatant, minding not to lose the pellet.
- 10. Add 250  $\mu$ L ethanol 70% to the pellet and homogenize gently the microtube twice without dissolving the pellet.
- 11. Centrifuge at 10,000 x g for 10 minutes at 4  $^{\circ}$
- 12. Remove the supernatant almost entirely, carefully not to loose the pellet. Leave the microtube open at room temperature for 5-10 minutes until complete evaporation of the ethanol 70%.

13. Resuspend the pellet in 20  $\mu$ L RNAse-free water and store the sample at -70  $^{\circ}$ C in the freezer.

OBS<sub>1</sub>: All extraction procedures must be developed using RNAse-free material (tips and microtubes). All solvents and solutions must be stored in dry flasks rinsed previously with DEPC and autoclaved. Solutions such as ethanol 70% must be prepared with RNAse-free water DEPC treated.

 $OBS_2$ : For experiments done in 6 well plates or bottles, add 1 mL TRIzol or in a proportion according to description in the manual cited in the references. For 1 mL TRIzol, double the volume of the reagents in the extraction.

OBS<sub>3</sub>: The protocol above is designed towards the extraction of adherent cells. In the case of non-adherent cells RNA extraction, the steps 1 and 2 must be replaced by a procedure which includes the recovering of the cell suspension in a RNAse-free microtube, centrifuge to pellet the cells, removing the supernatant and then adding TRIzol to the cell pellet. Afterwards, incubate the sample for 5 minutes at room temperature and follow the protocol from step 3.

OBS<sub>4</sub>: After step 2, if the RNA extraction is not done right after recovering TRIzol from the cultures, the microtubes must be placed on dry ice immediately and then stored at -70°C in the freezer until the extraction moment.

OBS<sub>5</sub>: During the isopropanol precipitation step, the precipitation time might be extended to overnight incubation or ever farther, as long as it is kept at  $-70^{\circ}$ C in the freezer.

OBS<sub>6</sub>: When drying the pellets, mind not to leave it extremely dry, because it may hamper dissolving it.

OBS<sub>7</sub>: The discard of TRIzol must be done into red sacs of biological waste and stored in a cardboard can specific for phenol (must not be autoclaved).

OBS<sub>8</sub>: If the TRIzol reagent be substituted for other equivalent from another manufacturer, the specific manual must be consulted in order to perform the procedures

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http://tools.thermofisher.com/content/sfs/manuals/trizol\_reagent.pdf

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## **Protocol**