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Working

Adapted protocol to extract total RNA using TRIzol® (Invitrogen, Thermo) [↗](#)

PLOS One

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## ABSTRACT

Total RNA extraction using TRIzol reagent.

Attention: for nasopharyngeal aspirates, we use regular TRIzol reagent in this assay, but for blood samples it is better to use a special one as "TRIzol LS".

## EXTERNAL LINK

<https://doi.org/10.1371/journal.pone.0217744>

## THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Matsuno AK, Gagliardi TB, Paula FE, Luna LKS, Jesus BLS, Stein RT, Aragon DC, Carlotti APCP, Arruda E (2019) Human coronavirus alone or in co-infection with rhinovirus C is a risk factor for severe respiratory disease and admission to the pediatric intensive care unit: A one-year study in Southeast Brazil. PLoS ONE 14(6): e0217744. doi: [10.1371/journal.pone.0217744](https://doi.org/10.1371/journal.pone.0217744)

- 1 In a microfuge tube: 750ul of TRIzol (Invitrogen, Thermo)+ 250ul of sample
- 2 Incubate the homogeneized sample for 5min at r.t.
- 3 Add 150ul of chloroform
- 4 Shake the tube vigorously by vortex for 15sec.
- 5 Incubate for 3min at r.t.
- 6 Centrifuge for 15min at 12,000 x g and 4°C.
- 7 The mixture separates into red lower phenol-chloroform phase, and interphase, and a colorless upper aqueous phase. RNA remains in the upper phase while the DNA and proteins in the interphase.

- 8 Place the aqueous phase for a new microfuge tube.RNA IsolationRNA precipitation
- 9 Add 375ul of Isopropanol.
- 10 Homogeneize by vortex.
- 11 Incube for 1h at r.t.
- 12 Centrifuge for 30min at 12,000 x g and 4°C.
- 13 Discard the supernatant.RNA wash
- 14 Add 750ul of Ethanol 75%.
- 15 Homegeneize by vortex.
- 16 Centrifuge for 5min at 7,500 x g and 4°C.
- 17 Discard the supernatant.
- 18 Ressuspend the RNA pellet in 30uL of RNase-free H2O at 55-60°C.
- 19 Homogeneize for 5 min by vortex.
- 20 Store the RNA at -70°C.



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