JUN 04, 2019

Adapted protocol to extract total RNA using TRIzol® (Invitrogen, Thermo)

PLOS OneTalita B Gagliardi¹¹University of Maryland, College Park

Talita B Gagliardi

University of Maryland, College Park

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".

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.w8vfhw6

External link:

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

Protocol Citation: Talita B Gagliardi 2019. Adapted protocol to extract total RNA using TRIzol® (Invitrogen, Thermo). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.w8vfhw6>

MANUSCRIPT CITATION:

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)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: Jan 17, 2019

Last Modified: Jun 04, 2019

PROTOCOL integer ID:
19445

- 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 Isolation RNA 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** Homogenize by vortex.
- 16** Centrifuge for 5min at 7,500 x g and 4°C.
- 17** Discard the supernatant.
- 18** Resuspend the RNA pellet in 30uL of RNase-free H₂O at 55-60°C.
- 19** Homogenize for 5 min by vortex.
- 20** Store the RNA at -70°C.