

Organoid RNA extraction protocol

- 1) Remove media from organoids, replace with cell recover solution* (at least 350ul/well) and place on ice for at least 15 minutes (as many wells as desired, but should be at least 2-3).
- 2) Resuspend and pool each well in a 2ml Eppendorf tube (or larger centrifuge tube to accommodate larger volumes). Centrifuge 1250 rpm, 5 min, 4°C.
- 3) Remove as much supernatant as possible and replace with chilled PBS.
- 4) Repeat steps 2 and 3 at least 2 times.
- 5) Resuspend organoids with 1ml Trizol reagent. Pipette up and down several times to mix and homogenize cells.
- 6) Incubate room temperature for 5 min.
- 7) Add 0.2ml Chloroform per 1ml Trizol.
- 8) Secure the lid tightly and shake vigorously for 10-15 seconds.
- 9) Incubate for 2-3 minutes at RT.
- 10) Centrifuge for 15min, 12,000 x g, at 4°C.
- 11) Gently collect the clear-aqueous upper layer and transfer to a fresh RNase-free 1.5 ml Eppendorf tube. Be careful not to disturb the red-phenol-chloroform lower layer, or white precipitate in the interphase.
- 12) Add 1ul of 20mg/ml RNase free glycogen.
- 13) Add 0.5ml isopropanol .
- 14) Incubate at -20°C for at least 1 hour or overnight.
- 15) Centrifuge for 15 minutes, 12,000 x g, at 4°C.
- 16) Discard the supernatant.
- 17) Wash the pellet in 1ml cold 75% ethanol and centrifuge for 5 minutes, 12,000 x g, at 4°C.
- 18) Repeat #17.
- 19) Discard the supernatant with a pipette and air dry for 5-10 minutes. Do not dry entirely.
- 20) Resuspend in 50ul RNase free dH2O.
- 21) Treat with RNase-free DNase I using manufacturer protocols.
- 22) Repeat steps 5-20.
- 23) Quantify Final RNA concentration with a nano-drop or bioanalyzer and store at -80°C.

* Cell recovery solution is a proprietary mix that is used to degrade Matrigel. Corning Product #354253. [https://catalog2.corning.com/LifeSciences/en-US/Shopping/ProductDetails.aspx?productid=354253\(Lifesciences\)](https://catalog2.corning.com/LifeSciences/en-US/Shopping/ProductDetails.aspx?productid=354253(Lifesciences))