untitled protocol

Yuan Yao

Abstract

Citation: Yuan Yao untitled protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.qafdsbn
Published: 22 May 2018

Materials

DNase I (RNase-free) - 1,000 units M0303S by New England Biolabs

RNAse-free Water by Contributed by users

RNaseZap® AM9780 by Thermo Scientific

Protocol

Step 1.
Remove growth media.

Step 2.
Add 1 mL of TRIzol™ Reagent per 1 × 105—107 cells directly to the culture dish to lyse the cells.

Step 3.
Pipet the lysate up and down several times to homogenize.

Step 4.
Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.

Step 5.
Add 0.2 mL of chloroform or 50 µL of 4-bromoanisole per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.

Step 6.
Incubate for 2–3 minutes.

Step 7.

Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.

Step 8.
Transfer 600 µL of the colorless, upper aqueous phase containing the RNA to a new tube.

Step 9.
Add an equal volume of 70% ethanol, then mix well by vortexing.

Step 10.
Invert the tube to disperse any visible precipitate that may form after adding ethanol.

Step 11.
Transfer up to 700 uL of the sample to a spin cartridge (with collection tube).

Step 12.
Centrifuge at 12,000 × g for 15 seconds.

Step 13.
Discard the flow-through, then reinsert the spin cartridge into the same collection tube.

Step 14.
Repeat step 2a–step 2c until the entire sample has been processed.

Step 15.
Add 700 µL of Wash Buffer I to the spin cartridge.

Step 16.
Centrifuge at 12,000 × g for 15 seconds.

Step 17.
Discard the flow-through, then reinsert the spin cartridge into the same collection tube.

Step 18.
Add 500 µL of Wash Buffer II to the spin cartridge.

Step 19.
Centrifuge at 12,000 × g for 15 seconds.

Step 20.
Discard the flow-through, then reinsert the spin cartridge into the same collection tube.

Step 21.

Repeat step 18-step 20 once.

Step 22.
Centrifuge at 12,000 × g for 1 minute to dry the membrane.

Step 23.
Discard the collection tube, then insert the spin cartridge into a recovery tube.

Step 24.
Add 30 µL-3 × 100 µL (3 sequential elutions with 100 µL each) of RNase-free water to the center of thespin cartridge.

Step 25.
Incubate for 1 minute.

Step 26.
Centrifuge at >12,000 × g for 2 minutes.

Step 27.
Discard the spin cartridge.

Step 28.
RNA integrity was verified by electrophoresis on a 1.2% agarose gel containing formaldehyde, and post stained with 1.0 µg/ml ethidium bromide.

Step 29.
Determine the quality and quantity of the purified RNA using UV absorbance at 260 nm.