Protocol RNA isolation of Caco-2 cells based on the Maxwell® 16 LEV simplyRNA Tissue Kit from Promega

1. Prepare the Homogenization Solution: add 20 µL of 1-Thioglycerol per millilitre of Homogenization Solution.
2. **Note:** 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement.
3. **Note:** A volume of 200 µl of 1-Thioglycerol/Homogenization Solution is needed for each sample.
4. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.
5. Prepare DNase I as described in the Promega quick protocol.
6. Prepare cartridges. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack with the label side facing away from the Elution Tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
7. **Note:** If you are processing fewer than 16 samples, center the cartridges on the platform
8. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube
9. Place 0.5ml Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube
10. Thaw the Caco-2 cell pellets on ice
11. Add 200µl of chilled 1-Thioglycerol/Homogenization Solution to the cell pellet and vortex until pellet is dispersed and cells appear lysed. A pipette may be used to disperse the pellets before vortexing. Store lysed cells on ice if there is a delay before processing
12. Shortly before processing samples on the Maxwell® 16 Instrument, add 200µl of Lysis Buffer (Part# MC501C) to 200µl of lysed cells. Vortex vigorously for 15 seconds to mix
13. Transfer all 400µl of lysate to well #1 of the Maxwell® 16 LEV Cartridge (MCE). Well #1 is the closest to the cartridge label and farthest from the elution tube
14. Add 5µl of DNase I solution to well #4 (yellow reagent). After adding the blue DNase I solution, the reagent in well #4 will be green
15. Run samples on the Maxwell® 16 Instrument by selecting “RNA”, then select “simplyRNA” on the Menu screen
16. After instrument run, remove excessive beads by using a magnetic tube rack
17. Assess the RNA concentration and purity on the Nanodrop