# Total RNA Isolation Using TRIzol Reagent

By Hongwei Cheng, 2-17-03; commented by TCH

1. Remove media completely (**wash cells with PBS is optional, but usually unnecessary, in some cases it could hurt**).
2. Lyse the cells in T25 flask by adding 1ml TRIzol reagent directly (**3ml TRIzol reagent for T-75 flask, and other reagents are scaled up three-fold**). Swish/tilt the flask for one minute, make sure that the solution coats entire surface of the flask. Then use sterile cell scraper to pool all the lysate in a bottom corner, or just let the lysate flow down to a corner by tilting the flask for a while (**If necessary, cell scrapers can be used to collect lysate completely**). Transfer them into 2-ml RNase-free Eppendorf tubes (**e.g., freshly opened boxes or RNA Use Only boxes, <u>not the ones from your bench</u>!**). **Keep tubes on ice**.
3. Add 270ul chloroform (**i.e., 0.27ml chloroform per 1ml TRIzol reagent**), vortex vigorously.
4. Spin the sample at top speed in microfuge for 15 min **in cold room**. Transfer the colorless upper aqueous phase into a new set of 1.5-ml tubes (**Avoid touching the junks in interphase! Keep tubes on ice**).
5. Add 670ul of isopropanol (i.e., about 2/3 vol. of TRIzol reagent). **To maximize RNA recovery rate, you can add 3-5ul of <u>Glycogen</u> (but not seeDNA as it will interfere with your A260 reading later) to each tube**. Mix well (**Tubes can be kept at -80° for several days or weeks**).
6. Spin at top speed for 10 min in **cold room** to precipitate RNA.
7. Remove the supernatant. Wash the RNA pellet with 600ul of 75% ethanol.
8. Dissolve RNA in 50ul RNase-free water (**The typical yield from a confluent T-25 is about 50 to 100ugs**). Read A260/A280 and/or check RNA integrity on RNA agarose gel. Store RNA stock at –80ºC.

**<u>REAGENTS REQUIRED, BUT NOT SUPPLIED</u>**:

**Chloroform; isopropanol; 75% ethanol; and RNase-free ddH2O.**