(RNA extraction)

Materials：

cells

TRLZOL

chloroform

RNA-free water

Autoclaved tip, eppendrof tube

NucleoSpin RNA Clean-up XS (MACHEREY-NAGEL)

 Clean-up Buffer RCU 5ml Add 15ml 96～100% ethanol. It can be stored at RT for a year.

 Wash Buffer RA3 6ml Add 24mL 96～100% ethanol. It can be stored at RT for a year.

Phenol-Choloroform / Isoamyl alchohol Aqua layer and organic layer become transparent.

1. Wipe the desk with 70% Ethanol
2. Aspirate the medium of the cells in 10cmdish(55cm2), Add 8ml of TRIZOL Reagent 8ml and passing the cell lysate several times through a pipette to lyse the cells.

※The amount of TRIZOL Reagent added is based on the area of the culture dish (1 ml (200µl) per 10 (1.88) cm2 and not on the number of cells present. An insufficient amount of TRIZOL Reagent may result in contamination of the isolated RNA with DNA.

1. Incubate the homo for 5min at RT.
2. Transfer it into 15ml tube, divide it into 8 1.5ml eppendrof tube each 1ml. Store it at －80℃.
3. Add 0.2ml (40µl) of chloroform per 1 ml (200µl) of TRIZOL, and vortex it for 15 sec.
4. Incubate it for 3 min at RT.
5. Centrifuge it at 14000rpm(12000×g), 4℃ for 30min.
6. Following centrifugation, the mixture　separates into a lower red, phenol-chloroform phase (DNA, protein), an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase.
7. Transfer the aqueous phase to a fresh tube.



1. Adjust RNA binding conditions :Add one volume of Buffer RCU to the sample (e.g.100µl RCU to 100µl sample) and vortex it for 5s 2 times (5×2s). Spin it down for 1s at 1000×g.
2. Bind RNA: Take one NucleoSPin RNA XS Column (light blue ring) placed in a Collection Tube (2ml) for each preparation. Load up to 300 µl sample mix to the column. Centrifuge for 30s at 14000rpm(11,000×g). For volumes exceeding 300 μL, load the sample mix in subsequent centrifugation steps onto the column. Place the column in a new Collection tube (2ml).
3. Wash and dry silica membrane: Add 400 µl Buffer RA3 to the NucleoSpin RNA XS Column. Centrifuge for 30s at 14000rpm. Discard flowthrough and pace the column back into the Collection Tube. Add 200 µl Buffer RA3 to the NucleoSpin RNA XS column. Centrifuge for 2 min at 14000 rpm to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5mL, supplied).
4. Elute RNA: Elute the RNA in 10µl RNase-free H2O, (supplied) and centrifuge at 14000rpm for 30s.