Nama Gene Azma

NamaZol RNA Extraction Protocol

NamaZol reagent contains phenol and guanidine thiocyanate. This reagent is toxic and can also cause burns if it touches the skin.
Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at -70°C or in liquid nitrogen until RNA isolation.
Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.

1- **A:** For tissue samples (5-50mg), add 750µl **NamaZol** reagent and homogenize using a homogenizer.

   B: For whole blood sample add 750µl **NamaZol** reagent to 250µl sample and pipette up and down to lysis cells.

2- Incubate the homogenized sample for 5-15 min at RT.
3- Add 200µl chloroform to each sample, and shake it vigorously for 15 sec, then incubate at RT for 2-5 min.
4- Centrifuge tube at 12000 rpm for 15 min at 4°C.
5- Transfer the colorless upper aqueous phase to a new microtube.
6- Add 500µl isopropanol to the aqueous phase, then invert it several times to mix it thoroughly. Incubate sample for 5-10 min at RT.
7- Centrifuge the sample at 12000 rpm for 10 min at 4°C.
8- Discard the supernatant and add 1ml, 75% ethanol to each microtube. Vortex briefly.
9- Centrifuge the sample at 8000 rpm for 5 min at 4°C.
10- Discard the supernatant and remove the excess ethanol from the RNA pellet by air-drying.
11- Resuspend the RNA pellet in 20-100µl DEPC-treated water and incubate for 10-15 min at 55°C. Extracted RNA should be stored at -70°C.