Nama Gene Azma

RNA Extraction W.B/Tissue 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 or quick-freeze samples after collection and store at -70°C or in liquid nitrogen until RNA isolation. Use nuclease-free water (DEPC-Treated water) to prepare the reagents.

Add 40 ml absolute ethanol to Wash Buffer II.

1- Add 750µl **NamaZol** reagent to 250µl sample.

A: Tissue samples (5-50mg), homogenize using a homogenizer.

B: Whole blood sample (250µl) pipette up and down to completely lyse cells.

Incubate the homogenized samples for 5-15 min at RT.

2- Add 200µl chloroform to each sample, and shake it vigorously for 15 sec, then incubate at RT for 2-5 min.
3- Centrifuge microtubes at 12000 rpm for 15 min at 4°C.
4- Transfer the colorless upper aqueous phase to a new microtube.
5- Add 100µl isopropanol and 400µl **Binding Buffer** to the sample then mix thoroughly.
6- Add the sample to the spin column in one or two steps then centrifuge for 1 min at 10000 rpm.
7- Place the column in a new collection tube and add 500µl **Wash Buffer II**.
8- Centrifuge for 1 min at 10000 rpm. Then proceed centrifugation at full speed (14000 rpm) for 2-3 min to dry the membrane completely.
9- Place the column in a clean 1.5ml microtube. Open the lid of the column and apply 20-100µl preheated **Elution Buffer** to the center of the membrane.
10- Close the lid and incubate at 65°C for 2-5 min. Centrifuge at full speed (>12000 rpm) for 1 min.
11- Store eluted RNA at -70°C.