14. septenber 2020

Special Protocol: Extarction of sperm RNA by use of FATRK kit

Additional requirement to be provided by user

1. Microcentrifuge capable of speed at ~12,000 rpm
2. 1.5 ml microcentrifuge tube
3. NaCl (0.9%)
4. ß-Mercaptoethanol
5. 2M NaOAc, pH 5.2.
5. Ethanol (70 %, RNase-free)
7. Staturated phenol
8. Chlorofrom
9. Vortex

1. Add 1.5 ml of 0.9 % NaCl to a microcentrifuge tube. (not provided)
2. Add 20 µl of sperm sample and invert the tube 5 times. Centrifuge the tube at 2,000 x g for 5 min and discard the supernatant.

   -- Note! Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.
3. Add 1.5 ml of 0.9 % NaCl to the sperm pellet and Invert the tube 5 times. Centrifuge the tube at 2,000 x g for 1 min and discard the supernatant.
4. Add 500 µl of FARB Buffer and 5 µl of ß-Mercaptoethanol to the sperm pellet. Homogenize the sample by using a rotor - stator homogenizer for 1 mn than incubate at room temperature for 10 min.
5. Add 50 µl 2M NaOAc, pH 5.2 and invert the tube 5 times.
6. Add 450 µl phenol (ddH2O saturated) and 100 µl chloroform into the tube, vortex vigorously for 2 minutes.
7. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase into a clean tube.
8. Add 1volume of 70 % ethanol and mix well by vortexing.
9. Place a FARB Mini Column to a Collection Tube and transfer up to 700 µl the sample mixture to the FARB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FARB Mini Column back to the Collection Tube.

10 Repeat the step 9 for the rest of the sample mixture.

11. Add 500 µl of Wash Buffer 1 to the FARB Mini Column, centrifugeat at full speed for 1 min.
    Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
12. Add 750 µl of Wash Buffer 2 to the FARB Mini Column, centrifuge at full speed for 1 min.
    Discard the flow-through and return the FARB Mini Column back to the Collection Tube.

    -- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.
13. Repeat step 9 for one more washing.
14. Centrifuge the FARB Mini Column at full speed for an additional 3 min to dry the FARB Mini Column.

    -- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
15. Place the FARB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).

16 Add 30 of RNase-free ddH2O to the membrane center of the FARB Mini Column. Stand the FARB Mini Column for 1 min.

    -- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.

    -- Important : Do not elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will lower the RNA yield.
17. Centrifuge the FARB Mini Column at full speed for 1 min to elute RNA.
18. Store RNA at -70C.6