RNA purification with FastPure® Cell/Tissue Total RNA Isolation Kit

Aim of the experiment

The purpose of this experiment is to purify RNA using FastPure® Cell/Tissue Total RNA Isolation Kit.This kit can quickly extract total RNA from animal cells or tissues. The kit is based on silica gel column purification technology. There is no need to use toxic phenol/chloroform extraction during the extraction process. The whole process only takes 30 minutes. The gDNA-Filter Columns in the kit can effectively separate the supernatant and adsorb and remove gDNA; RNAPure Columns can efficiently bind RNA and are equipped with optimized Buffer solution, so that the total RNA obtained is of high purity and free of protein and other impurities.

Materials

- Buffer RL1: Provides the environment needed for animal tissue and cell lysis;
- Buffer RL2: Provides a specific column environment for RNA;
- Buffer RW1: Removal of impurities such as protein and DNA in RNA;
- Buffer RW2: Remove salt residues in RNA;
- RNase-free ddH2O: eluting the total RNA on the purification column membrane;
- Buffer RDD: Provide the buffer environment needed for DNase I digestion;
- DNase I, RNase-free: remove the residue of gDNA;
- gDNA-Filter Columns: DNA adsorption column, and remove lysis impurities;
- RNAPure Columns: specific adsorption of RNA;
- Collection Tubes 2 ml: Filtrate collection tube;
- RNase-free Collection Tubes 1.5 ml: RNA collection tube.

Procedure

(The experiment was carried out in a fume hood without RNase contamination)

1. Transfer the bacterial solution to gDNA-Filter Columns (gDNA-Filter Columns have been placed in the collection tube), and centrifuge at 12,000 rpm (13,400 × g) for 2 min. Discard the gDNA-Filter Columns and save the supernatant in the collection tube.

2. Add 1.6 times the volume of Buffer RL2 (alcohol has been added) to the supernatant in the collection tube, and mix gently.

3. Transfer the mixture from step 2 to RNAPure Columns (RNAPure Columns have been placed in the collection tube), centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the waste solution.

4. After discarding the waste liquid, put the RNA Pure Columns adsorption column back into the collection tube, and add all the remaining liquid to the adsorption column.

5. Centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the waste liquid.

6. Add 500 μl Buffer RW1 to RNAPure Columns, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the waste solution.

7. Add 700 μl Buffer RW2 (alcohol has been added) to RNAPure Columns, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the waste solution.

8. Put the RNAPure Columns adsorption column back into the collection tube, and centrifuge at 12,000 rpm (13,400 × g) for 2 minutes to completely remove the ethanol residue in RNAPure Columns.

9. Transfer the adsorption column to a new RNase-free Collection Tubes 1.5 ml centrifuge tube, and add 50-200 μl of RNase-free ddH2O to the center of the adsorption column. Leave it at room temperature for 2 min, and centrifuge at 12,000 rpm (13,400 × g) for 1 min to elute RNA.

10. The extracted Total RNA can be used directly in downstream experiments or stored at -80°C.