FastPure Cell/Tissue Total RNA Isolation Kit

RC101

Version 8.1

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Introduction

FastPure Cell/Tissue Total RNA Isolation Kit provides a fast and simple technique for preparing total RNA from animal cells or tissues. Based on the technology of purification of silica gel column, this kit is achieved without the use of phenol/chloroform extractions, which makes the RNA isolation procedure simple and can be performed in less than 30 minutes. gDNA-Filter Columns in the kit can effectively separate the supernatant and substantially reduce genomic DNA contamination; RNAPure Columns can efficiently bind RNA, along with optimized Buffer solution make the isolation of total RNA with high purity, free of protein and other impurities, and can be used in various downstream experiments such as RT-PCR, Real-Time PCR and chip analysis.

Components

Components	RC101-01 (50 rxn)
Buffer RL1	30 ml
Buffer RL2	15 ml
Buffer RW1	60 ml
Buffer RW2	36 ml
RNase-free ddH_2O	10 ml
Buffer RDD	6 ml
DNase I，RNase-free	250 μl
gDNA-Filter Columns (each in a 2 ml Collection Tube)	50 Tubes
RNAPure Columns (each in a 2 ml Collection Tube)	50 Tubes
RNase-free Collection Tubes 1.5 ml	50 Tubes

Buffer RL1: Provides the environment needed for animal tissue and cell lysis;
Buffer RL2: Provides an RNA-specific on column environment;
Buffer RW1: removes impurities such as proteins and DNA;
Buffer RW2: removal of salt ion residues;
RNase-free ddH_2O: elutes the total RNA on the column membrane;
Buffer RDD: Provides the buffering environment required for DNase I digestion;
DNase I, RNase-free: removal of gDNA residues;
gDNA-Filter Columns: DNA adsorption column, and remove lysis impurities;
RNAPure Columns: adsorb of RNA specifically;
Collection Tubes 2 ml: filtrate collection tube;
RNase-free Collection Tubes 1.5 ml: RNA collection tube.

Storage Conditions

Store DNase I at -20℃;

Buffer RL1 can be stored at 4℃ for 1 month after adding β-mercaptoethanol;

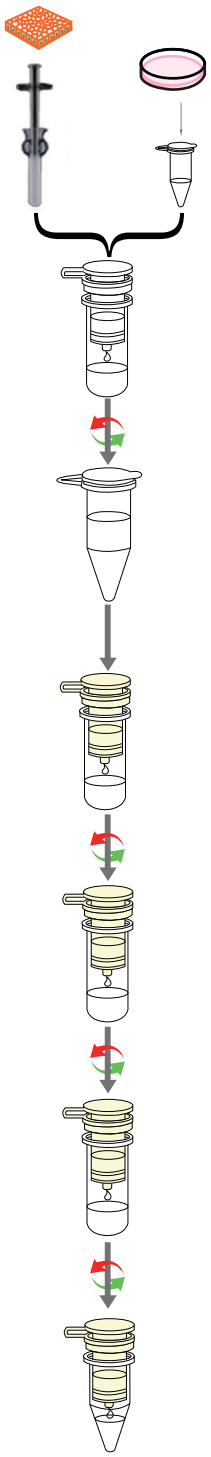
The remaining components can be stored under normal temperature (15 - 25℃) in drying conditions.

Experimental principle and process summary

Step one:

Animal tissue: 10 - 20 mg

Tissue homogenate: 500 μl Buffer RL1



Step one:

Collect cells: 2 - 5 x 10^6 cells

Lysis cells: 500 μl Buffer RL1

Step two:

Transfer the tissue homogenateor cell lysate to gDNA-Filter Columns, centrifuge at 13,000 × g for 2 min, and collect the filtrate.

Step three:

Add 1.6 times the volume of Buffer RL2 to the collected filtrate

Step four:

Add the mixture above to the RNAPure Colume, centrifuge at 13,000 × g for 1 min, discard the waste water.

Step five:

Remove protein: Add 500 μl of Buffer RW1 (centrifuge at 13,000 × g for 1 min), discard the waste

Desalting: Add 700 μl Buffer RW2 (centrifuge at 13,000 × g for 1 min), discard the waste

Step six (optional):

Remove gDNA residue: add 70 μl of DNase I to the center of the membrane, let stand at room temperature for 15 - 30 min, repeat step five

Step seven:

Add 700 μl of Buffer RW2 (centrifuge at 13,000 × g for 1 min) Removal of residual ethanol: centrifuge the empty tube at 13,000 × g for 2 min

Step eight:

Elution: add 50 - 200 μl RNase-free ddH_2O Incubate at room temperature for 2 - 5 min, centrifuge at 13,000 × g for 2 min, collect RNA

Protocol

Please read this instruction carefully before use. This kit is applicable for the extraction of total RNA from animal cells and tissues. Please follow the procedure carefully. This kit has been tested rigorously. Please do not change the adding amount of reagents. Add β-mercaptoethanol to Buffer RL1 and make it to a final concentration of 1% (10 μl β-mercaptoethanol per 1 ml Buffer RL1). This solution is recommended to use right after it was ready.

1. Sample Preparation

◇ Animal tissue

Homogenization: Add 500 µl of Buffer RL1 (with β-mercaptoethanol) to every 10 - 20 mg of fresh tissue, and thoroughly grind the tissue with a glass homogenizer or an electric homogenizer.

Grinding with liquid nitrogen: Immediately transfer the powder in liquid nitrogen to Buffer RL1 (β-mercaptoethanol has been added), add 500 µl Buffer RL1 per 10 - 20 mg, pipet evenly to no obvious powder mass.

▲ Add β-mercaptoethanol to Buffer RL1 to a final concentration of 1% (for example, add 10 µl of β-mercaptoethanol to 1 ml Buffer RL1) before use.

▲ The amount of tissue sample do not exceed 20 mg. Do not exceed 10 mg of DNA/RNA-rich samples such as liver, spleen, kidney, etc., otherwise the quality of total RNA extraction will decrease.

▲ Make sure that the temperature does not exceed 25℃ during the homogenization process; high temperature will cause total RNA degradation.

▲ if it is not immediately subjected to the next step, the prepared sample can be stored at -70℃.

◇ Cultured cells

Adherent cells: do not need to digest, can be directly digested and lysed by Buffer RL1 (contain β-mercaptoethanol); or trypsinized and centrifuged to collect cells and add Buffer RL1 (contain β-mercaptoethanol), add 500 µl of Buffer RL1 to every 2 - 5×10^6 cells. Pipette repeatedly to mix thoroughly until no cell mass is visible.

Suspension cells: Collect cells by direct centrifugation, add Buffer RL1 (contain β-mercaptoethanol), add 500 µl of Buffer RL1 to every 2 - 5×10^6 cells, pipette repeatedly to mix thoroughly until no cell mass is visible.

▲ Add β-mercaptoethanol to Buffer RL1 to a final concentration of 1% (for example, add 10 µl of β-mercaptoethanol to 1 ml Buffer RL1) before use.

▲ Add 500 µl of Buffer RL1 to every 2 - 5 x 10^6 cells.

▲ If the next step is not performed immediately, the cell lysate can be stored at -70℃.

2. RNA Purification

The following procedure should be carried out in an RNase-free clean bench.The following procedure should be carried out in an RNase-free clean bench.

1. Transfer the treated mixture to a gDNA-Filter Columns (gDNA-Filter Columns have been placed in the collection tube) and centrifuge at 13,000 × g for 2 min. Discard the gDNA-Filter Columns and retain the supernatant in the collection tube.
   ▲ If the homogenization or grinding is not complete and the tissue usage amount is greater than 20 mg, transfer the homogenate to a 1.5 ml centrifuge tube, centrifuge at 13,000 x g for 5 min, and transfer the supernatant to gDNA-Filter Columns and centrifuge at 13,000 x g for 2 min.
   ▲ If there is a precipitate at the bottom of the collection tube of gDNA-Filter Columns, transfer the supernatant to a new RNase-free tube for further operation. Do not ingest the sediment into the supernatant.
2. Add 1.6 times the supernatant volume of Buffer RL2 (with absolute ethanol added) to the supernatant in the collection tube and mix gently.
   ▲ The volume of the supernatant is generally 500 µl. Add 800 µl Buffer RL2. The amount of Buffer RL2 should be added according to the volume of the supernatant during the actual operation.
   ▲ If there is turbidity or precipitate appears in the solution after Buffer RL2 added , it is normal. You can directly proceed to the next step after pipetting.
3. Transfer mixture from the step 2 to RNAPure Columns (RNAPure Columns have been placed into the collection tube), centrifuge at 13,000 × g for 1 min, and discard the effluent.
   ▲ The volume of the adsorption column is 700 µl. Please centrifuge twice.
   ▲ Mix the flocculent precipitates appearing in step 2 and transfer them to RNAPure Columns to centrifuge.
4. After discarding the waste liquid, put the RNAPure Columns back into the collection tube, add all the remaining liquid to the adsorption column, centrifuge at 13,000 × g for 1 min, and discard the waste liquid.
5. Add 500 µl of Buffer RW1 to the RNAPure Columns, centrifuge at 13,000 × g for 1 min, and discard the waste.
6. Add 700 µl of Buffer RW2 (with absolute ethanol) to the RNAPure Columns, centrifuge at 13,000 × g for 1 min, and discard the waste.
7. Trace DNA removal (optional):
   a.Preparation of DNase I reaction working solution: Add 65 µl Buffer RDD to the RNase-free centrifuge tube and add 5 µl of DNase I, mix gently.
   b.Add 70 µl of DNase I reaction solution to the center of the membrane and let stand for 15 - 30 min at room temperature.
   c.Add 500 µl of Buffer RW1 to the RNAPure Columns, centrifuge at 13,000 × g for 1 min, and discard the waste water.
   d.Add 700 µl of Buffer RW2 to the RNAPure Columns, centrifuge at 13,000 × g for 1 min, and discard the waste water.

8. Repeat step 6.
9. Place the RNAPure Columns column back into the collection tube and centrifuge at 13,000 × g for 2 min to completely remove the Buffer RW2 remaining in the RNAPure Columns.
10. Transfer the column to a new 1.5 ml RNase-free Collection Tubes and add 50 - 200 µl of RNase-free ddH_2O to the center of the column. Place it at room temperature for 2 min and centrifugation at 13,000 × g for 1 min to elute RNA.
    ▲ The elution volume of RNase-free ddH_2O should not be lower than 50 µl. Too small a volume will affect the elution efficiency. To increase total RNA production, please pre-warm RNase-free ddH_2O at 65℃, re-add the RNA eluate to the RNAPure Columns, placed it at room temperature for 2 min, centrifuged at 13,000 × g for 1 min, and discard RNAPure Columns.
11. The purified total RNA can be used directly in downstream experiments or stored at -70℃.

ISO 9001: 2015