TIANGEN®

DNA/RNA Isolation Kit

For simultaneous purification of genomic DNA and total RNA from the same animal cells or tissues

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DNA/RNA Isolation Kit

(Spin Column)

Cat.no. 4992456

Kit Contents

Contents	4992456 50 preps
Buffer RLplus	30 ml
Buffer RW	12 ml
Buffer RW1	40 ml
RNase-Free ddH_2O	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
RNase-Free Columns CR3 Set	50
Spin Columns CB3	50
RNase-Free Centrifuge Tubes 1.5 ml	100
RNase-Free Centrifuge Tubes 2 ml	50
RNase-Free Collection Tubes 2 ml	50
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Compatible Reagents

DNase I (Cat. no. 4992232)

Storage

Buffer RLplus added with β-mercaptoethanol could stay 30 days at 2-8°C. All the other reagents should be stored in dry place and at room temperature (15-30°C) for 15 months.

Introduction

DNA/RNA Isolation Kit is designed for extracting both genomic DNA and total RNA simultaneously from the same animal cells or tissue samples. This kit is compatible with a wide range of animal cells and tissues. The simple process allows the purification of high-quality DNA and RNA from the same sample within 40-50 min. The purified DNA and RNA are eluted separately and ready-to-use in downstream applications.

Notes of preventing RNase contamination

1. Wear gloves when handling RNA and all reagents, as skin is a common source of RNase. Change gloves frequently.
2. Use RNase-Free certified, disposable plastic ware and filter tips whenever possible.
3. Buffer RLplus could protect RNA. But for experiment, RNA should be stored or applied in RNase-Free plastic or glassware. To wipe off RNase, the glassware could be dried at 150°C for 4 hours, while plastics could be dipped in 0.5 M NaOH for 10 min, and washed by RNA-Free ddH_2O thoroughly and sterilized.
4. Use RNase-Free ddH_2O to prepare solution (RNase-Free ddH_2O: add 0.1 ml DEPC to 100 ml ddH_2O and shake vigorously to bring DEPC into solution. Let the solution stand overnight. Autoclave to remove any trace of DEPC).

Important Notes

1. Add β-mercaptoethanol (β-ME) to Buffer RLplus to a final concentration of 1% before use. For example, add 10 µl β-mercaptoethanol (β-ME) per 1 ml Buffer RLplus. Buffer RLplus may form precipitate during storage. If necessary, redissolve by warming it in 56°C, and then equilibrate to room temperature.
2. Before start, add ethanol (96-100%) to Buffer RW, Buffer PW and Buffer GD for a working solution, as described on the bottle.
3. The following operations were carried out at room temperature unless otherwise indicated.
4. For some sensitive RNA samples, genomic DNA may need to be removed completely in the f ollowing application. Please refer to DNase I digestion process with column.

Protocol

Simultaneous Purification of Genomic DNA and Total RNA from Cultured Cells

1. Cell harvest:

1a. Cells grown in suspension (do not use more than 1 x 10^7 cells):

Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x g in a centrifuge tube. Carefully remove all supernatant by aspiration, and proceed to step 2.

1b. Cells grown in a monolayer (do not use more than 1 x 10^7 cells):

Cells grown in a monolayer in cell-culture vessels could be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

1). To lyse cells directly: Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.

2). To trypsinize and collect cells: Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10-0.25% trypsin into PBS. After the cells detach from the dish or flask, add medium(containing serum to inactivate the trypsin), then transfer the cells to an RNase-Free glass or polypropylene centrifuge tube (not supplied), and centrifuge for 5 min at 300 x g. Completely aspirate the supernatant, and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the combination of RNA and spin column, resulting in the reduction of RNA yield.

2. Cell lysis:

For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of Buffer RLplus (Ensure that β-ME is added to Buffer RLplus before use) according to table 1, vortex to mix for 30 sec.

Table 1 Volumes of Buffer RLplus for Lysing Pelleted Cells

Number of pelleted cells	Volume of Buffer RLplus
<5 x 10^6	350 µl
5 x 10^6- 1 x 10^7	600 µl

For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RLplus (Ensure that β-ME is added to Buffer RLplus before use) according to table 2. Collect the lysate into a microcentrifuge tube, vortex to mix for 30 sec.

Table 2. Volumes of Buffer RLplus for Direct Cell Lysis

Dish diameter	Volume of Buffer RLplus
<6 cm	350 µl
6-10 cm	600 µl

3. Pipet the lysate directly into a spin column CB3 placed in a 2 ml collection tube, and centrifuge for 2 min at 12,000 rpm (~13,400 x g) to collect the filtrate. Place the spin column CB3 into the collection tube at room temperature or 4°C for later DNA purification.

Total RNA purification

4. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the flow-through obtained from step 3, and mix well by pipetting. Transfer the mixture (including any precipitate that may have formed) to an RNase-Free Spin Column CR3 placed in a 2 ml collection tube, centrifuge for 2 min at 12,000 rpm (~13,400 x g). Discard the flow-through.

   Note: Use RNase-Free ddH_2O when preparing 70% ethanol. If the filtrate volume is lost, please reduce the amount of ethanol by 70%. When the solution and the precipitate are transferred to the spin column CR3, if the volume is larger than the adsorption column capacity, it can be completed in two steps.
5. Add 700 µl Buffer RW1 to Spin Column CR3. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g) to wash the spin column membrane. Discard the flow-through. Put Spin Column CR3 back to the collection tube.
6. Add 500 µl Buffer RW (Ensure that ethanol is added before use) to the RNase-Free Spin Column CR3. Close the lid gently, incubate for 2 min at room temperature and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g). Discard the flow-through. Put Spin Column CR3 back to the collection tube.

7. Repeat step 6.
8. Centrifuge for 2 min at 12,000 rpm (~13,400 x g). Discard the flow-through. Dry the Spin Column CR3 at room temperature for a few minute to clean up Buffer RW totally.

   Note: The purpose of this step is to remove the residual rinsing liquid in the spin column. After centrifugation, the spin column CR3 is left at room temperature for a while to dry sufficiently. If there is residual rinsing liquid, it may affect downstream experiments such as reverse transcription.
9. Place the RNase-Free Spin Column CR3 in a new 1.5 ml collection tube (supplied). Add 100 µl RNase-Free water directly to the spin column membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 12,000 rpm (~13,400 x g) to elute the RNA.

   Note:Elution buffer volume should be at least 30 µl or that will lead to low yield of RNA purification. RNA eluted should be stored at -70°C.

Genomic DNA purification

10. Add 500 µl Buffer GD (Ensure that ethanol is added before use) to the Spin Column CB3 from step 3. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g) to wash the spin column membrane. Discard the flow-through.
11. Add 500 µl Buffer PW (Ensure that ethanol is added before use) to the Spin Column CB3. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g) to wash the spin column membrane. Discard the flow-through.
12. Repeat step 11.
13. Centrifuge for 2 min at 12,000 rpm (~13,400 x g). Discard the flow-through. Dry the Spin Column CB3 at room temperature for a few minutes to clean up Buffer PW totally.

    Note: The purpose of this step is to remove the residual rinsing liquid in the spin column. After centrifugation, the spin column CB3 is left at room temperature for a while to dry sufficiently. If there is residual rinsing liquid, it may affect downstream experiments.
14. Place the Spin Column CB3 in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer TB directly to the spin column membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 12,000 rpm (~13,400 x g) to elute the DNA.

Simultaneous Purification of Genomic DNA and Total RNA from Animal Tissues

1. Sample disruption and homogenization:
   Disrupt the tissue and homogenize the lysate in Buffer RLplus (Ensure that β-ME is added to Buffer RLplus before use) according to table 3, using a rotor-stator homogenizer. Vortex to mix for 30 sec.

Table 3. Volumes of Buffer RLplus for Tissue Disruption and Homogenization

Amount of starting material	Volume of Buffer RLplus
10-20 mg	350 µl
≥20 mg	600 µl

   Note: Do not use over 30 mg, otherwise RNA yield and quality will be reduced.
2. Centrifuge the lysate for 3-5 min at 12,000 rpm (~13,400 x g). Carefully remove the supernatant by pipetting, and transfer it to the Spin Column CB3 placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g) to collect the filtrate. Place the Spin Column CB3 into the collection tube at room temperature or 4°C for later DNA purification.

Total RNA purification

3. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the flow-through from step 2, and mix well by pipetting (Precipitates may be visible after addition of ethanol, but this does not affect the procedure).
   Note: Ensure 70% ethanol is made of RNase-Free water; reduce the volume if there is a loss of the filtrate.
4. Transfer up the sample, including any precipitate that may have formed, to a Spin Column CR3 placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g). Discard the flow-through.
   Note: If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same column.
5. Add 700 µl Buffer RW1 to the Spin Column CR3. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g). Discard the flow-through.

6. Add 500 µl Buffer RW (Ensure that ethanol is added before use) to the Spin Column CR3. Incubate for 2 min, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g). Discard the flow-through.
7. Repeat step 6.
8. Centrifuge for 2 min at 12,000 rpm (~13,400 x g). Discard the flow-through. Place Spin Column CR3 at room temperature for 2 min to clean up the Buffer RW totally.
   Note: Ensure that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions such as reverse transcription.
9. Place the Spin Column CR3 in a new 1.5 ml collection tube. Add 30-100 µl RNase-Free water directly to the spin column membrane. Incubate for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 x g) to elute the RNA.
   Note:Elution buffer volume should be at least 30 µl, otherwise RNA yield and quality will be reduced. RNA eluted should be stored at -70°C.

Genomic DNA purification

10. Add 500 µl Buffer GD (Ensure that ethanol is added before use) to the Spin Column CB3 from step 2. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g). Discard the flow-through.
11. Add 500 µl Buffer PW (Ensure that ethanol is added before use) to the Spin Column CB3. Close the lid gently, incubate for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 x g). Discard the flow-through.
12. Repeat step 11.
13. Centrifuge for 2 min at 12,000 rpm (~13,400 x g) and discard the flow-through. Place Spin Column CB3 at room temperature for 2 min to clean up the Buffer PW totally.
    Note: Ensure that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.
14. Place the Spin Column CB3 in a new 1.5 ml collection tube (supplied). Add 30-100 µl Buffer TB directly to the spin column membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 12,000 rpm (~13,400 x g) to elute the DNA.

DNase I digestion procedure (optional)

Preparation of DNase I stock solution: Dissolve the lyophilized DNase I (1500 units) in 550 µl of the RNase-Free ddH_2O. Mix gently by inverting. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months.

Note: Thawed aliquots could be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

1. Follow the procedure of RNA purification step 1-4.
2. Add 350 µl Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through.
3. Preparation of DNase I working solution: Add 10 µl DNase I stock solution (see Preparation of DNase I stock solution) to 70 µl Buffer RDD. Mix by gently inverting the tube.
4. Add 80 µl DNase I working solution directly to the RNase-Free Spin Column CR3, and place on the bench top for 15 min.
5. Add 350 µl Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through.
6. Follow the procedure of RNA purification step 6-9.