TIANGEN®

DNA/RNA/Protein Isolation Kit

For simultaneous purification of genomic DNA, total RNA, and total protein from the same cell or tissue sample

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DNA/RNA/Protein Isolation Kit

(Spin Column)

Cat. no. 4992729

Kit Contents

Contents	4992729 50 preps
Buffer RL	30 ml
Buffer RW1	40 ml
Buffer RW	12 ml
RNase-Free ddH_2O	15 ml
RNase-Free Spin Columns CR3 Set	50
Spin Columns CB3	50
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
RNase-Free Centrifuge Tubes (1.5 ml)	100
RNase-Free Centrifuge Tubes (2 ml)	50
RNase-Free Collection Tubes (2 ml)	50
Buffer PR	220 ml
Buffer SP	15 ml
Handbook	1

Compatible Reagents

DNase I (Cat. no. 4992232)

Storage

Buffer RL added with β-mercaptoethanol could be stored for 30 days at 4°C. All the other reagents should be stored dry at room temperature (15-25°C) and is stable for at least 12 months.

Introduction

This Kit is designed to purify genomic DNA, total RNA, and total protein simultaneously from a single biological sample (cultured cells and animal tissues) and allows the parallel processing of multiple samples.

RNA Protection

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 RL could protect RNA. But for experiment, RNA should be stored or applied in RNase-Free plastic or glassware. To inactivate 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 Before Starting

1. Add β-mercaptoethanol (β-ME) to Buffer RL to a final concentration of 1% before use. For example, add 10 µl β-mercaptoethanol (β-ME) per 1 ml Buffer RL. Buffer RL may form precipitate during storage. If necessary, redissolve by warming it at 56°C, and then equilibrate to room temperature.
2. Before use, add ethanol (96-100%) to Buffer RW, Buffer PW and Buffer GD for the working solution, as described on the tag.
3. Perform all steps of the procedure at room temperature (15-25°C) if not emphasized.
4. For some sensitive RNA samples, genomic DNA may need to be removed completely in the following application. Please refer the on-column DNase I digestion procedure.

Protocol

Simultaneous Purification of Genomic DNA and Total RNA from Cultured Cells

1. Cell harvest :

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

   Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 × g in a centrifuge tube. Carefully remove all supernatant by aspiration, and proceed to step2.

   Cells grown in a monolayer (do not use more than 1×10^7 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 step2.

   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 × g. Completely aspirate the supernatant, and proceed to step2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

2. Lyse the cells by adding Buffer RL:

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

Volumes of Buffer RL for Lysing Pelleted Cells

Number of pelleted cells	Volume of Buffer RL (μl)
$<5\times 10^6$	350
5×10^6- 1×10^7	600

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

Volumes of Buffer RL for Direct Cell Lysis

Dish diameter (cm)	Volume of Buffer RL (μl)
<6	350
6-10	600

3. Pipet the lysate directly into a Spin Column CB3 placed in a 2ml collection tube, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g) to collect the filtrate. Place the Spin Column CB3 into a 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 centrifuge tube (not supplied), centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Transfer the flow-through for protein purification to a 2 ml collection tube (supplied). Place RNase-Free Spin Column CR3 back to the collection tube.

   Note: Prepare 70% ethanol using RNase-Free ddH_2O. Please reduce the volume of 70% ethanol if there is loss of flow-through obtained from step 3. If the sample volume exceeds 700 μl, centrifuge successive aliquots in the same column.

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×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 12,000rpm (~13,400 × 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 × g). Discard the flow-through. Dry the Spin Column CR3 at room temperature for a few minutes to clean up Buffer RW totally.

   Note: Ensure that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

9. Place the RNase-Free Spin Column CR3 in a new 1.5 ml centrifuge tube (supplied). Add 100 µl RNase-Free ddH_2O directly to the spin column membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

   Note: Elution buffer volume should be over 30 µl, otherwise that will lead to low yield of RNA purification. RNA eluate should be stored at -70°C.

Genomic DNA purification

10. Add 500 µl Buffer GD to the Spin Column CB3 **(ensure that ethanol is added before use).** Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g) to wash the spin column membrane. Discard the flow-through.
11. Add 500 µl Buffer PW to the Spin Column CB3 **(ensure that ethanol is added before use).** Close the lid gently, incubate for 2 min at the room temperature and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × 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 × g). Discard the flow-through. Dry the Spin Column CB3 at room temperature for a few minutes to clean up 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 centrifuge tubes (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 × g) to elute the DNA.

Please refer to page 8 for protein purification.

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 RL **(according to table as below, ensure that β-Mercaptoethanol (β-ME) has been added before use)** using a rotor-stator or a glass homogenizer. Vortex to mix for 30 sec.

   Volumes of Buffer RL for Tissue Disruption and Homogenization

Amount of starting material (mg)	Volume of Buffer RL (µl)
10-20	350
>=20	600

   Note: Do not use over 30 mg of tissue, otherwise RNA yield and quality will be reduced.

2. Centrifuge the lysate for 3-5 min at 12,000 rpm (~13,400 × g). Carefully remove the supernatant by pipetting, and transfer it to the Spin Column CB3 placed in a 2 ml RNase-Free centrifuge tube. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g) to collect the filtrate. Place the Spin Column CB3 into a collection tube at room temperature or 4°C for later DNA purification.

Total RNA purification

3. Add 1 volume (usually 350µl or 600µl) of 70% ethanol to the filtrate, and mix well by pipetting (precipitates may be visible after addition of ethanol, but this does not affect the procedures).

   Note: Ensure 70% ethanol is prepared with RNase-Free ddH_2O; reduce the volume if there is a loss of the filtrate.

4. Transfer the sample, including any precipitate that may have formed, to a Spin Column CR3 placed in a 2 ml RNase-Free centrifuge tube. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Keep the flow-through for protein purification and put Spin Column CR3 back to the 2 ml collection tube.

   Note: If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same column.

5. Add 700µl Buffer RW1 to the Spin Column CR3. Close the lid gently, and centrifuge for 30-60sec at 12,000 rpm (~13,400 × g). Discard the flow-through and put the RNase-Free Spin Column CR3 back to the collection tube.
6. Add 500 µl Buffer RW to the Spin Column CR3 **(ensure that ethanol is added before use).** Incubate for 2 min at room temperature, and centrifuge for 30-60 sec at 12,000rpm (~13,400 × g). Discard the flow-through.
7. Repeat step 6.
8. Centrifuge for 2 min at 12,000 rpm (~13,400 × 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.
9. Place the Spin Column CR3 to a new 1.5 ml centrifuge tube. Add 100 µl RNase-Free ddH_2O directly to the spin column membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

   Note: Elution buffer volume should be over 30 µl, otherwise RNA yield and quality will be reduced. RNA eluate should be stored at -70°C.

Genomic DNA purification

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

    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 centrifuge 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 × 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 -20°C for up to 9 months.

Note: Thawed aliquots could be stored at 4°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 (20-30°C) 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 procedures of RNA purification step 6-9.

Total protein precipitation

1. Add 4 volume of cool acetone (not supplied) or Buffer PR to the filtrate from step 4 in RNA purification. Mix thoroughly and put on ice or at -20°C for 10-30 min (the tube is not supplied).

   Note: The protein precipitate obtained using acetone is difficult to dissolve but the protein is higher in content than that using Buffer PR. Please choose the appropriate solution according to specific experiment.
2. Centrifuge for 10 min at 4°C at 12,000 rpm (~13,400 × g). Discard the supernatant.

3. Add 100 µl of 95% cool ethanol to the protein pellet. Centrifuge at full speed for 1 min, and remove the supernatant using pipet as much as possible.
4. Dry the protein pellet at room temperature.

   Note: Incomplete drying may cause problems when loading the protein onto a gel due to residual ethanol. Excessive drying makes protein difficult to dissolve.
5. Add 100 µl or appropriate volume of Buffer SP and mix vigorously to dissolve the protein pellet. The volume of Buffer SP used depends on the amount of starting material and the downstream experiment.

   Note: Protein obtained from Buffer SP could be used in SDS-PAGE and Western blot, but not in Bradford protein assay. If the protein needs to be quantified by Bradford protein assay, 5% SDS should be used to dissolve the protein, or select a buffer compatible with the intended downstream application.

SDS-PAGE procedure

6. Add protein loading buffer to the sample and incubate for 5-10 min at 95°C to completely dissolve and denature protein. Then cool the sample to room temperature.
7. Centrifuge for 1 min at full speed to pellet any residual insoluble material. Use the supernatant in downstream applications such as SDS-PAGE and western blot. The dissolved protein can be stored at -20°C for several months or at 4°C for several days.