TIANGEN®

# RNAprep Pure Cell/Bacteria Kit

For purification of total RNA from cultured animal cell and bacteria

# RNAprep Pure Cell/Bacteria Kit

**(Spin Column)**

**Cat. no. GDP430**

## Kit Contents

| | Contents | GDP430<br>50 preps |
|---|---|---|
| GDP430H | Buffer RL | 30 ml |
| | Buffer RW1 | 40 ml |
| | Buffer RW | 12 ml |
| | RNase-Free dd$H_2O$ | 15 ml |
| | RNase-Free Columns CR3 set | 50 |
| | RNase-Free Columns CS set | 50 |
| | RNase-Free Centrifuge Tubes (1.5 ml) | 50 |
| | Handbook | 1 |
| GRT411 | RNase-Free DNase I (1500 U) | 1 |
| | Buffer RDD (DNA Digest Buffer) | 4 ml |
| | RNase-Free dd$H_2O$ | 1 ml |

**Note: GDP430H, GRT411 are shipped and packaged separatly.**

## Compatible Reagents

Lysozyme (for bacteria RNA purification)

## Storage

RNase-Free DNase I, Buffer RDD and RNase-Free dd$H_2O$ (Tubular) should be stored at 2-8°C for 15 months; Buffer RL/β-Mercaptoethanol mix can be stored at 2-8°C for 1 month; others stored at room temperature (15-30°C) for 15 months.

## Introduction

RNAprep Pure Cell/Bacteria Kit provides a fast, simple, and cost-effective method for purification of total RNA from cultured animal cells and bacteria. The purified RNA is ready for use in downstream applications such as: RT-PCR and real-time RT-PCR, gene-chips assay, northern blot, dot blot, polyA screening, in vitro transcript, and molecular cloning.

## Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic and tips to avoid cross contamination.
3. RNA can be protected in Buffer RL. But RNA must be stored or applied in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10 min, washed by RNase-Free dd$H_2O$ thoroughly, and sterilized.
4. Use RNase-Free dd$H_2O$ to confect solution.

## Yield of total RNA with RNAprep Pure Cell/Bacteria Kit

| Source | Maximum number of cells | Yield of total RNA ($1x10^6$) |
|---|---|---|
| COS | $3 \times 10^6$ | Up to 35 μg |
| Hela | $7 \times 10^6$ | Up to 15 μg |
| NIH/3T3 | $10 \times 10^6$ | Up to 10 μg |

## Important points before starting

1. β-Mercaptoethanol (β-ME) must be added to Buffer RL before use. The final concentration of β-ME is 1%. For example, add 10 μl β-ME to 1 ml Buffer RL. Buffer RL containing β-ME can be stored at 2-8°C for 1 month. Buffer RL may form a precipitate upon storage. If necessary, re-dissolve by warming, and then place at room temperature (15-30°C).
2. Buffer RW is supplied as a concentrate. Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.
3. Perform all steps in RT if not indicated, centrifugation steps at 15-30°C in a standard centrifuge.

**Preparation of DNase I stock solution**

Dissolve the lyophilized DNase I (1500 U) in 550 µl of the RNase-Free dd$H_2O$. Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months.

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

## Protocol

**Purification of total RNA from cultured animal cells**

1. Harvest cells:

   a. 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 (not supplied). Carefully remove all supernatant by aspiration.

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

   Cells can be either lysed directly in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lyses. Cells grown 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 in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-Free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 × g for 5 min. Aspirate the supernatant completely.

   **Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the spin column membrane. Both effects may result in lower RNA yield.**

2. Disrupt 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 **(see the table as below; ensure that β-Mercaptoethanol has been added to Buffer RL before use).**

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

For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RL **(see the table as below, ensure that β-Mercaptoethanol has been added to Buffer RL before use)**. Pipet the lysate into a centrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

Volumes of Buffer RL for Direct Cell Lysis

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

3. Pipet the entire lysate, including any precipitate that may have formed, to an RNase-Free Spin Column CS placed in a 2 ml RNase-Free Collection Tube (supplied). Close the lid gently, and centrifuge for 2 min at 12,000 rpm (~13,400 x g).
4. Add 1 volume of 70% ethanol (usually 350 µl or 600 µl) to the cleared lysate, and mix immediately by pipetting. Transfer the sample, including any precipitate that may have formed, to RNase-Free Spin Column CR3 placed in a 2 ml RNase-Free Collection Tube. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.

   **Note: please confect 70% ethanol with RNase-Free dd$H_2O$. If sample is lost partly, reduce accordingly the amount of 70% ethanol; if lysate volume exceeds 700 µl, centrifuge successive aliquots in the same spin column.**
5. Add 350 µl Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
6. 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. Inverting the tube gently to mix.
7. Add the DNase I working solution (80 µl) directly to the center of RNase-Free Spin Column CR3, and place on the bench top for 15 min.

8. Add 350 µl Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
9. Add 500 µl Buffer RW to the RNase-Free Spin Column CR3 (**Ensure that ethanol has been added to Buffer RW before use**). Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) to dry the spin column membrane.

    **Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.**
12. Place the RNase-Free Spin Column CR3 in a new 1.5 ml RNase-Free Collection Tube (supplied). Add 30-100 µl RNase-Free dd$H_2O$ directly to the spin column membrane. Close the lid gently, place at room temperature (15-30°C) for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

    **Note: If the volume of elution buffer is less than 30 µl, it may affect recovery efficiency. Purified RNA is recommended be stored at -70°C.**

## Purification of total RNA from bacteria

1. Collect bacteria by centrifuging for 12,000 rpm (~13,400 × g) at 4°C in a centrifuge tube (do not use more than 1 x $10^9$ cells). Carefully remove all supernatant by aspiration, and proceed to step 2. Other steps should be completed at RT (15-30°C).

   **Note: non-complete removal of culture medium will inhibit lysis and dilute the lysate.**
2. Re-suspend bacteria thoroughly with 100 µl TE buffer containing lysozyme (recipe and incubation time have been showed as follow).

| | Concentration of lysozyme in TE buffer | Incubation time (RT) |
|---|---|---|
| G- bacterium | 400 µg /ml | 3-5 min |
| G+ bacterium | 3 mg/ml | 5-10 min |

3. Add 350 µl Buffer RL **(Ensure that β-Mercaptoethanol has been added to Buffer RL before use)**. Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 4. If insoluble precipitate

has been formed, centrifuge for 2 min at 12,000 rpm (~13,400 × g). Transfer supernatant to another centrifuge tubes.

4. Add 250 µl ethanol to the cleared lysate, mix immediately by pipetting. Transfer the sample, including any precipitate that may have formed, to RNase-Free Spin Column CR3 placed in a 2 ml RNase-Free Collection Tube. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through, and put the spin column back to the Collection Tube.
5. Add 350 µl Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
6. 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. Inverting the tube gently to mix.
7. Add the DNase I working solution (80 µl) directly to the center of RNase-Free Spin Column CR3, and place on the bench top for 15 min.
8. Add 350 µl Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
9. Add 500 µl Buffer RW to RNase-Free Spin Column CR3 **(Ensure that ethanol has been added to Buffer RW before use)**. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) to dry the spin column membrane.

    **Note: long centrifugation could dry the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.**
12. Place the RNase-Free Spin Column CR3 in a new 1.5 ml RNase-Free Collection Tube (supplied). Add 30-100 µl RNase-Free dd$H_2O$ directly to the spin column membrane. Close the lid gently, place at room temperature (15-30°C) for 2 min and then centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

    **Note: If the volume of elution buffer is less than 30 µl, it may affect recovery efficiency. Purified RNA is recommended be stored at -70°C.**