

# 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 ddH <sub>2</sub> O        | 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 ddH <sub>2</sub> O        | 1 ml               |

**Note:** GDP430H, GRT411 are shipped and stored separately.

## Compatible Reagents

Lysozyme (self provide, TIANGEN, Cat. no GRT401, for bacteria RNA purification)

## Storage

RNase-Free DNase I, Buffer RDD and RNase-Free ddH<sub>2</sub>O (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 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 ddH<sub>2</sub>O thoroughly, and sterilized.
4. Use RNase-Free ddH<sub>2</sub>O to confect solution.

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

| Source  | Maximum number of cells | Yield of total RNA (1x10 <sup>6</sup> ) |
|---------|-------------------------|-----------------------------------------|
| COS     | 3 × 10 <sup>6</sup>     | Up to 35 µg                             |
| Hela    | 7 × 10 <sup>6</sup>     | Up to 15 µg                             |
| NIH/3T3 | 10 × 10 <sup>6</sup>    | 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.
4. Do not recommend storage sample in buffer with lysing capacity.

### Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 U) in 550  $\mu$ l of the RNase-Free ddH<sub>2</sub>O. 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  $1 \times 10^7$  cells):

Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300  $\times$  g in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration.

b. Cells grown in a monolayer (do not use more than  $1 \times 10^7$  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  $\times$  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  $\beta$ -Mercaptoethanol has been added to Buffer RL before use**).

| Number of pelleted cells                  | Volume of Buffer RL (μl) |
|-------------------------------------------|--------------------------|
| < 5 x 10 <sup>6</sup>                     | 350                      |
| 5 x 10 <sup>6</sup> - 1 x 10 <sup>7</sup> | 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                      |

- 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 × g).
- 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 confirm 70% ethanol with RNase-Free ddH<sub>2</sub>O. 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.**

- 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.
- 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.
- 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, place in room temperature for 2 min and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, and then discard the waste liquid. Place the RNase-Free Spin Column CR3 at room temperature for 2-5 mins to completely dry the residual washing buffer in the column.  
**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 ddH<sub>2</sub>O 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 × 10<sup>9</sup> 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  $\mu$ l 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 ( $\sim 13,400 \times g$ ). Discard the flow-through, and put the spin column back to the Collection Tube.
5. Add 350  $\mu$ l Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through.
6. Preparation of DNase I working solution: Add 10  $\mu$ l DNase I stock solution (see Preparation of DNase I stock solution) to 70  $\mu$ l Buffer RDD. Inverting the tube gently to mix.
7. Add the DNase I working solution (80  $\mu$ l) directly to the center of RNase-Free Spin Column CR3, and place on the bench top for 15 min.
8. Add 350  $\mu$ l Buffer RW1 to the RNase-Free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through.
9. Add 500  $\mu$ l Buffer RW to RNase-Free Spin Column CR3 (**Ensure that ethanol has been added to Buffer RW before use**). Close the lid gently, place in room temperature for 2 min and centrifuge for 30-60 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm ( $\sim 13,400 \times g$ ) for 2 min, and then discard the waste liquid. Place the RNase-Free Spin Column CR3 at room temperature for 2-5 mins to completely dry the residual washing buffer in the column.
12. Place the RNase-Free Spin Column CR3 in a new 1.5 ml RNase-Free Collection Tube (supplied). Add 30-100  $\mu$ l RNase-Free ddH<sub>2</sub>O 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 ( $\sim 13,400 \times g$ ) to elute the RNA.

**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.

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