EasyPrep Cell & Bacteria RNAprep Purification Kit

For purification of total RNA from cultured animal cells and bacteria

Contents

Introduction

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

Tips for 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 RBL. However, RNA must be stored in or used with RNase-free plastic or glassware. To remove RNase, glassware can be heated at 150 °C for 4 h and plastic can placed in 0.5 M NaOH for 10 min, washed with RNase-free ddH_2O thoroughly, and sterilized.
4. Use RNase-free ddH_2O to prepare solutions.

Total RNA yield with EasyPrep Cell & Bacteria RNAprep Purification Kit

Source	Yield of total RNA	Maximum number of cells
COS	Up to 35 μg	$3x10^6$
Hela	Up to 15 μg	$7x10^6$
NIH/3T3	Up to 10 μg	$10x10^6$

Important points before starting

1. β-Mercaptoethanol (β-ME) must be added to Buffer RBL before use. The final concentration of β-ME is 1% (add 10 μL of β-ME to 1 mL of Buffer RBL). Buffer RBL containing β-ME can be stored at 4 °C for 1 month. Buffer RBL may form a precipitate upon storage. If necessary, dissolve by warming and storing at room temperature (15–25 °C).
2. Buffer RBW is supplied as a concentrate. Before using, add 96%–100% ethanol (indicated on the bottle) to obtain a working solution (add 48 mL of ethanol to Buffer RBW).
3. Perform all steps in RT unless indicated otherwise and perform centrifugations at 20–25 °C in a standard microcentrifuge.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 U) in 550 μL of RNase-free ddH_2O. Mix gently by inverting. Do not vortex. Divide the mixture into single-use aliquots and store at −20 °C for up to 9 months. Thawed aliquots can be stored at 2–8 °C for up to 6 weeks. Do not refreeze the aliquots after thawing.

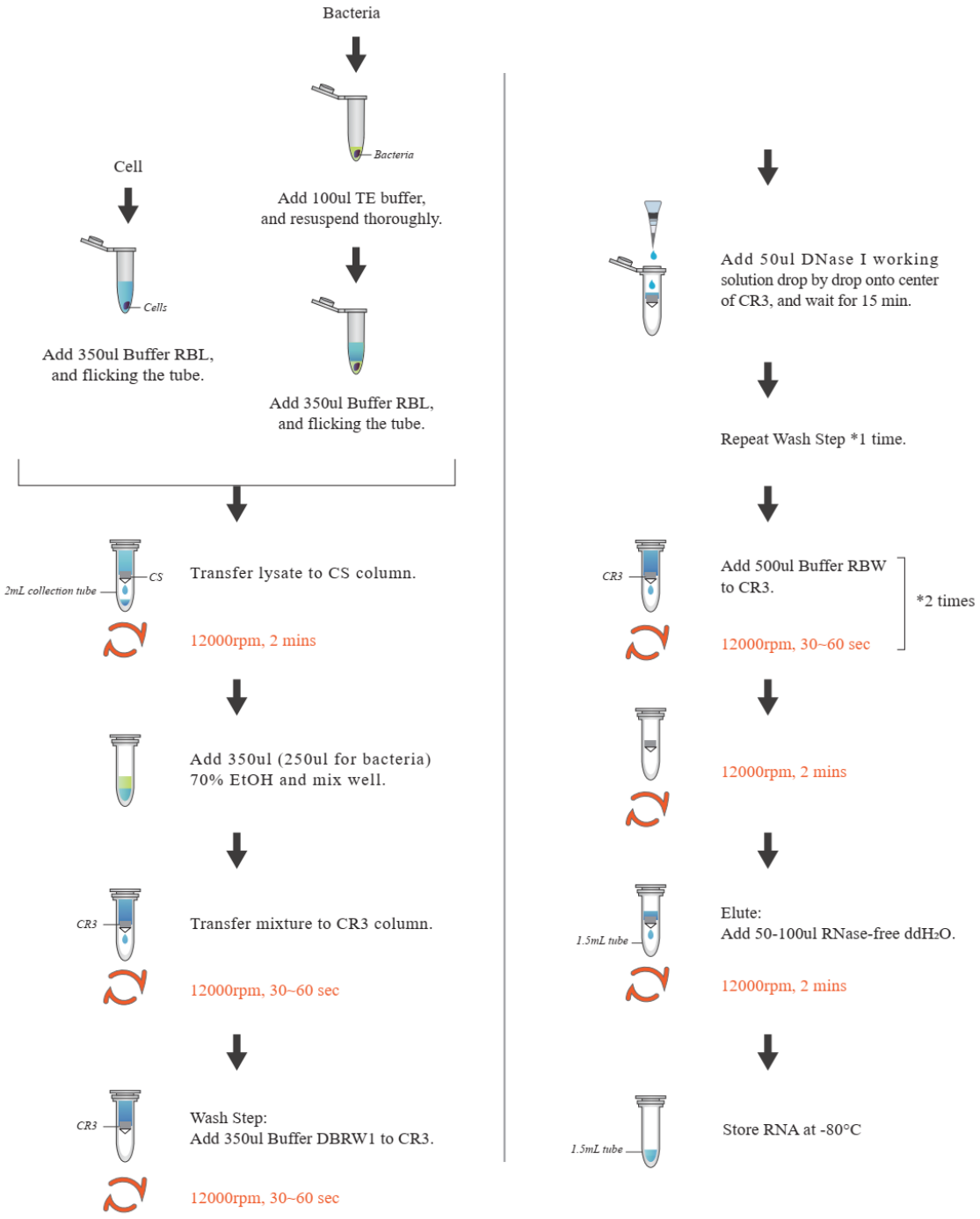
Kit Contents

Contents	DPT-BD30 (50 preps)
Buffer RBL	30 ml
Buffer DBRW1	40 ml
Buffer RBW	12 ml
DNase I（1500 U）	1
Buffer RBDD	4 ml
RNase-free ddH_2O（Tubular）	1 ml
RNase-free ddH_2O（Bottled）	15 ml
RNase-free Spin Columns CR3 in 2 ml collection tubes	50
RNase-free Filtration Columns CS in 2 ml collection tubes	50
RNase-free Collection Tubes（1.5 ml）	50

Storage

RNase-free DNase I, Buffer RBDD, and RNase-free ddH_2O (Tubular) should be stored at 2–8 °C; Buffer RBL/β-mercaptoethanol mix can be stored at 4 °C for 1 month; the others can be stored at room temperature (15–25 °C).

Workflow


Bacteria
Bacteria
Cell
Add 100ul TE buffer,
and resuspend thoroughly.
Cells
Add 350ul Buffer RBL,
and flicking the tube.
Add 350ul Buffer RBL,
and flicking the tube.
2mL collection tube
CS
Transfer lysate to CS column.
12000rpm, 2 mins
Add 350ul (250ul for bacteria)
70% EtOH and mix well.
CR3
Transfer mixture to CR3 column.
12000rpm, 30~60 sec
CR3
Wash Step:
Add 350ul Buffer DBRW1 to CR3.
12000rpm, 30~60 sec
Add 50ul DNase I working
solution drop by drop onto center
of CR3, and wait for 15 min.
Repeat Wash Step *1 time.
CR3
Add 500ul Buffer RBW
to CR3.
*2 times
12000rpm, 30~60 sec
12000rpm, 2 mins
1.5mL tube
Elute:
Add 50-100ul RNase-free ddH2O.
12000rpm, 2 mins
1.5mL tube
Store RNA at -80°C

Protocol

Purification of total RNA from cultured animal cells

1. Harvest cells according to steps a or b.
   a. Cells grown in suspension (do not use more than 1×10^7 cells):
      Determine the number of cells. Pellet cells by centrifuging for 5 min at 300 × *g* in a centrifuge tube (not supplied). Carefully remove all supernatant through aspiration and proceed to step 2.
   b. Cells grown in a monolayer (do not use more than 1×10^7 cells):
      Cells can either be lysed directly in the cell-culture flask (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

To lyse cells directly

Determine the number of cells. Completely aspirate the cell-culture medium and proceed immediately to step 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 0.1%–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 and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for RNA binding to the membrane, which may result in a low RNA yield.

2. Disrupt the cells by adding Buffer RBL
   For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add an appropriate volume of Buffer RBL (see the table below; ensure that β-mercaptoethanol is added to Buffer RBL before use).

Number of pelleted cells	Volume of Buffer RBL (μl)
< $5x10^6$	350
$5x10^6$ - $1x10^7$	600

For the direct lysis of cells grown in a monolayer, add an appropriate volume of Buffer RBL (see the table below; ensure that β-mercaptoethanol is added to Buffer RBL before use). Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

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

Note: Optimization steps for lysis of cell samples. After adding Buffer RBL, use medium or high vortex to complete cell lysis, the cytoplasmic part will become transparent and thick if lysis is complete (cell volume >10^6, RNA release); if lysis is not complete, it will be a mist.

3. Transfer the entire lysate, including any precipitate that may have formed, to the 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).
4. Add 1 volume of 70% ethanol (usually 350 μL or 600 μL) to the 2-mL collection tube which contained flow-through of Spin Column CS in step 3, 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 flowthrough.

Note: Prepare 70% ethanol with RNase-free ddH_2O. If the sample is partially lost, reduce the amount of 70% ethanol accordingly; if the volume of the lysate is more than the maximum amount in the RNase-free Spin Column CR3, divide the lysate in two

5. Add 350 μL of buffer DBRW1 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 flowthrough.
6. Preparation of DNase I working solution: Add 10 μL of DNase I stock solution (see Preparation of DNase I stock solution) to 70 μL of buffer RBDD. Invert the tube gently to mix.
7. Add DNase I working solution (80 μL) directly to the center of the RNase-free Spin Column CR3 and place on the bench top for 15 min.
8. Add 350 μL of buffer DBRW1 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 flowthrough.
9. Add 500 μL of buffer RBW to the RNase-free Spin Column CR3 (ensure that ethanol is added to buffer RBW before use). Close the lid gently and centrifuge for 30–60 s at 12,000 rpm (~13,400 × *g*). Discard the flowthrough.
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 time 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 50–100 μL of RNase-free ddH_2O directly to the spin column membrane. Close the lid gently, store at room temperature (15–25 °C) for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × *g*) to

elute the RNA.

Note: If elution is performed in small volumes (<50 µL), the elution buffer must be dispensed on the center of the membrane for the complete elution of bound RNA.

Purified RNA may be stored at −80 °C

Purification of total RNA from bacteria

1. Collect bacteria by centrifugation at 12, 000 rpm (~13,400 × *g*) and 4 °C in a centrifuge tube (do not use more than 1×10^9 cells. For *Escherichia coli*, OD_{600} of 1.0 ≈ 8×10^8 cells/mL). Carefully remove all supernatant by aspiration, and proceed to step 2. Other steps should be completed at RT (20–25 °C).

Note: Incomplete removal of the culture medium will inhibit lysis and dilute the lysate.

2. Resuspend bacteria thoroughly with 100 µL of TE buffer containing lysozyme (recipe and incubation times are shown in table below).

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

3. Disrupt the cells by adding Buffer RBL. Loosen the cell pellet thoroughly by flicking the tube. Add 350 µL of Buffer RBL (ensure that β-mercaptoethanol is added to Buffer RBL before use) Vortex or pipet to mix and ensure that no cell clumps are visible before proceeding to step 4. If an insoluble precipitate forms, centrifuge for 2 min at 12,000 rpm (~13,400 × *g*). Transfer the supernatant to another microcentrifuge tube.
4. Add 250 µL of ethanol (95%–99%) to the cleared lysate and mix immediately by pipetting. Transfer the sample, including any precipitate that may have formed, to the 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 flowthrough.
5. Add 350 µL buffer DBRW1 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 flowthrough.
6. Preparation of DNase I working solution: Add 10 µL of DNase I stock solution (see Preparation of DNase I stock solution) to 70 µL of buffer RBDD. Invert the tube gently to mix.
7. Add the DNase I working solution (80 µL) directly to the center of the RNase-free Spin Column CR3 and leave it on the bench top (20–30 °C) for 15 min.
8. Add 350 µL buffer DBRW1 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 flowthrough.
9. Add 500 µL of buffer RBW to the RNase-free Spin Column CR3 (ensure that ethanol is added to buffer RBW before use). Close the lid gently and centrifuge for 30–60 s at 12,000 rpm (~13,400 × *g*). Discard the flowthrough.

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 time 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 of RNase-free ddH_2O directly to the spin column membrane. Close the lid gently, leave at room temperature (15–25 °C) for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × *g*) to elute the RNA.

Note: If elution is performed in small volumes (<50 µL), the elution buffer must be dispensed onto the center of the membrane for the complete elution of bound RNA. Purified RNA may be stored at −80 °C.