Cyrusbioscience

GeneKlean® Total RNA Isolation Kit-Cell/Bacteria/Blood/Fungus

Cat. Number: TRNACBBF

Storage: Store at Room Temperature.

Sample: Up to 10^7 of mammalian cells, 10^9 of bacteria cells or 300uL of whole blood.

Yield: Up to 30ug Total RNA

Kit Contents

Total RNA Isolation Kit-Plant	100 Reactions
GeneKlean® Total RNA Column with 2 mL Collection Tube	100pcs
Resuspension Buffer	110mL
Lysis Buffer	45mL
G(-) Bacteria Cells Resuspension Buffer	25mL
Wash Buffer 1	45mL
Wash Buffer 2	15mL
(add Ethanol)	(60mL)
Elution Buffer	10mL
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Note

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination.
- Add ethanol (>99.5%) to Wash Buffer 2, shake before use (see bottle label for volume).
- Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- Buffers contain irritants. Wear gloves when handling these buffers.

Additional requirements

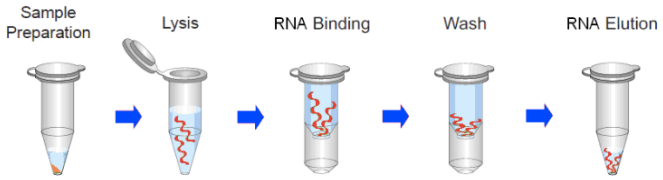
- Ethanol (>99.5%)
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- b-mercaptoethanol(14.3M)
- For Optional Step (DNA Residue Degradation): Add 2 µl DNAse I (2KU/ml) and 10 µl reaction buffer (300 mM Tris-HCl (pH 7.5), 60 mM MnCl2, 300 µg/ml BSA) to the 50µl final product. Let stand for 10 minutes at room temperature (at 25°C).

- For Gram-positive bacteria sample: **Lysozyme Buffer** (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use)
- For Fungus sample: Lyticase or Zymolase, **Sorbitol Buffer** (1.2 M sorbitol;10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

Description

GeneKlean® Total RNA Isolation Kit provides a fast, simple, and cost-effective method for isolation of total RNA from whole blood, mammalian cells and bacterial cells. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows RNA species bases to bind to the glass fiber matrix of the spin column. GeneKlean® Total RNA Isolation Kit is suitable for a variety of routine applications including RT-PCR, cDNA Synthesis, Northern Blotting, Differential display, Primer Extension and mRNA Selection. The entire procedure can be completed within 25-40 minutes.

Procedure for Preparation of Total RNA Isolation



Sample Preparation

Fresh Blood

1. Collect blood in EDTA-Na2 treated collection tubes(or other anticoagulant mixtures).
2. Transfer up to **300uL of blood** to a sterile 1.5 mL microtube.
3. Add **900uL of Resuspension Buffer** and mix by inversion.
4. Incubate the tube on ice for 10 min(invert twice during incubation).
5. Centrifuge at 4,000 x g for 5 min at 4°C. **Remove the supernatant completely** and resuspend the cells in **100uL of Resuspension Buffer** by pipetting the pellet up and down.

Cultured Mammalian Cells

1. Transfer cultured mammalian cells(up to 10^7) to a sterile 1.5 mL microtube.
2. Centrifuge at 6,000 x g for 1 min. **Remove the supernatant completely** and resuspend the cells in **100uL of Resuspension Buffer** by pipetting the pellet up and down.

G(-) Bacteria Cells

1. Transfer cultured bacteria cells(up to 10^9) to a sterile 1.5 mL microtube.

2. Centrifuge at 12,000 x g for 1 min. **Remove the supernatant completely** and resuspend the cells in **200uL of G(-) Bacteria Cells Resuspension Buffer** by pipetting the pellet up and down. Incubate at room temperature for 5 min.

G(+) Bacteria Cells

1. Transfer cultured bacteria cells(up to 10^9) to a sterile 1.5 mL microtube.
2. Centrifuge at 12,000 x g for 1 min. **Remove the supernatant completely** and resuspend the cells in **200uL of Lysozyme Buffer** by pipetting the pellet up and down. Incubate at room temperature for 10 min.

Fungus Cells

1. Transfer fungus cells(up to 10^8) to a sterile 1.5 mL microtube.
2. Centrifuge at 6,000 x g for 5 min. **Remove the supernatant completely** and resuspend the cells in **600uL of Sorbitol Buffer** by pipetting the pellet up and down.
3. Add **200U of Lyticase or Zymolase**. Incubate at 30°C for 30 min.
4. Centrifuge at 2,000 x g for 10 min to harvest the spheroplast. **Remove the supernatant completely** and resuspend the cells in **200uL of G(-) Bacteria Cells Resuspension Buffer** by pipetting the pellet up and down. Incubate at room temperature for 5 min.

Step 1. Lysis

Fresh Blood/Mammalian Cells

1. Add **400uL of Lysis Buffer** and 4uL of b-Mercaptoethanol to the resuspended cells from Sample Preparation Step and shake vigorously. Incubate at room temperature for 5 min.
2. Centrifuge at 16,000 x g for 10 min. Transfer the supernatant to a new 1.5 mL microtube

Bacteria Cells/Fungus Cells

1. Add **300uL of Lysis Buffer** and 3uL of b-Mercaptoethanol to the sample lysate from Sample Preparation Step and mix by vortex. Incubate at room temperature for 5 min.
2. Centrifuge at 16,000 x g for 10 min. Transfer the supernatant to a new 1.5 mL microtube

Step 2. RNA Binding

1. Add 500 µl of 70% ethanol prepared in ddH2O (RNase-free and DNase-free) to the sample lysate from Step 1 and shake vigorously (break up any precipitate by pipetting).
2. Place a GeneKlean® Total RNA Column in a 2mL Collection Tube. Apply 600uL of the mixture to GeneKlean® Total RNA Column.
3. Centrifuge at 15,000 x g for 1 minute. Discard the flow-through and place the GeneKlean® Total RNA Column in the same Collection tube. Transfer the remaining mixture to the same column.
4. Centrifuge at 15,000x g for 1 min. Discard the flow-through and place the Total RNA Column back to the same Collection Tube.

Step 3. Washing

1. Add 400uL of Wash Buffer 1 to the GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs. Discard the flow-through and place the Total RNA Column back to the same Collection Tube.
2. Add 600uL of Wash Buffer 2(ethanol added) into GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs. Discard the flow-through and place the GeneKlean® Total RNA Column back to the same Collection Tube.
3. Centrifuge again for 2 mins at 15,000x g to dry the column matrix.

Step 4. Elution

1. Place GeneKlean® Total RNA Column in a clean 1.5mL microtube.
2. Add 50uL of Elution Buffer to the center of GeneKlean® Total RNA Column, stand for 2 mins. Centrifuge 2 mins at 15,000x g.
   *Optional DNase treatments can be followed to remove unwanted DNA residue.

Troubleshooting

Problems	Possible Causes	Suggestions
Degraded RNA/Low integrity	RNase Contamination	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, Ex: RNase inhibitor.
Low yields of RNA	Incomplete lysis and homogenization	Use the appropriate method for the lysate preparation based on the amount of starting materials immersed in the Lysis Buffer to achieve the optimal lysis.
	Incorrect elution conditions	Add 100 uL of the Elution Buffer to the center of each Column, let it stand for 2 minutes, and centrifuge at 15,000 x g for 2 minutes.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Repeat the wash step: Centrifuge at 15,000 x g again for 2 minutes to remove the residual of Wash Buffer 2.