Product Specification

Product:	BB Total RNA Isolation Kit (Blood/Cultured Cell/Fungus)(Column Based)
Product Code:	BRIK00100
Sample:	Up to 300µl of Whole Blood, Up to 10^7 Mammalian cells, Up to 10^9 Bacterial cells, Up to 10^8 Fungus cells
Yield:	Up to 30µg
Elution volume:	50 - 200µl
Format:	Spin Column
Operation time:	Within 40mins

Catalog Number	**Size**
BRIK00100	100 reactions

Description

The BB Total RNA Isolation Kit (Blood/Cultured Cell/Fungus)(Column Based) provides a fast, simple, and cost-effective method for isolation of high-quality total RNA from tissue sample. 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 while contaminants pass through the column. Impurities are efficiently washed away, and the pure RNA is eluted with RE Buffer without phenol extraction or alcohol precipitation. RNA purified with BB Total RNA Isolation Kit is suitable for a variety of routine downstream applications including RT-PCR, cDNA Synthesis, Northern Blotting, Differential Display, Primer Extension and mRNA Selection. Consistent RNA yield using small amount of starting material. The entire procedure can be completed within 40mins.

Storage

Stable for up to 24 months at 18 - 25°C

Kit Content(s)	
Buffer RL	110ml
Buffer RA	45ml
Buffer RO	25ml
Buffer W1	45ml
Buffer W2 (Add ethanol)	15ml (60ml)
Buffer RE	10ml
DR Columns	100pcs
Collection Tubes	100pcs

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Required materials but not provided

- Absolute Ethanol (96 – 100%)
- 1.5ml microcentrifuge tubes
- RNase-free Pipette tips
- 14.3M ß-mercaptoethanol
- For optional step of DNA Residue Degradation: Add 2µl of the DNase I (2KU/ml) mixed in a reaction buffer {50mM Tris-HCl (pH 7.5) and 10mM $MnCl_2$, 50µg/ml BSA at 25ºC} to the final elution sample. Let it stand for 10mins at room temperature
- Lysozyme Buffer (for Gram-positive Bacteria)
  [20mg/ml lysozyme; 20mM Tris-HCl; 2mM EDTA; 1% Triton X-100; pH 8.0]
- Lyticase or Zymolase (for fungus)
- Sorbitol Buffer (for fungus)
  [1.2M sorbitol; 10mM $CaCl_2$; 0.1M Tris-HCl pH 7.5; 35mM β-mercaptoethanol]

Buffer Preparation

- Add 60ml of Absolute Ethanol (96 – 100%) to Buffer W2 and mix well before use

BB Total RNA Isolation Kit Protocol (Column Based)

[For Fresh Blood]

Step 1 – Sample Cells Harvesting

1. Collect blood in the EDTA-Na_2-treated Collection Tubes (or other anticoagulant mixtures)
2. Transfer up to 300µl of blood to a sterile 1.5ml microcentrifuge tube
3. Add 900µl of Buffer RL to the sample and mix by inversion
4. Incubate the tube on ice for 10mins (invert twice during incubation)
5. Centrifuge at 4,000 x g for 5mins at 4ºC. Remove the supernatant completely and resuspend the cells in 100µl of Buffer RL by pipetting the pellet

Step 2 – Lysis

1. Add 400µl of Buffer RA and 4µl of β-mercaptoethanol to the tube of resuspended cells in Step 1 and shake vigorously to mix. Incubate for 5mins at room temperature
2. Centrifuge at 16,000 x g for 10mins
3. Transfer the supernatant to a new 1.5ml microcentrifuge tube

Step 3 – RNA Binding

1. Add 500µl of 70% ethanol prepared with ddH_2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting)
2. Place a DR Column on a Collection Tube. Add 600µl of the mixture from the previous step to the DR Column
3. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube
4. Transfer the remaining mixture to the same DR Column
5. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube

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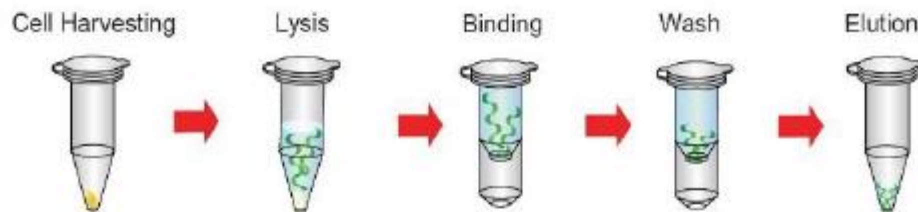
Step 4 – Wash

1. Add 400µl of Buffer W1 into the DR Column
2. Centrifuge at 14,000 x g for 30secs
3. Discard the flow-through and place the DR Column back onto the same Collection Tube
4. Add 600µl of Buffer W2 (Ethanol added) into the DR Column
5. Centrifuge at 14,000 x g for 30secs
6. Discard the flow-through and place the DR Column back onto the same Collection Tube
7. Centrifuge at 14,000 x g again for 2mins to remove the residual Buffer W2

Step 5 – RNA Elution

1. To elute RNA, place the DR Column onto a new clean 1.5ml microcentrifuge tube
2. Add 50 - 200µl of Buffer RE to the center of each DR Column, allow it to stand for 2mins
3. Centrifuge at 14,000 x g for 2mins to elute the purified RNA
   *Optional DNase treatments can be followed to remove unwanted DNA residue
   Note: Check buffers for salt precipitation before use. Re-dissolve any precipitate by warming to 37°C



[For Cultured Mammalian Cells]

Step 1 – Sample Cells Harvesting

1. Transfer the cultured Mammalian cells (up to 10^7) to a sterile 1.5ml microcentrifuge tube
2. Centrifuge at 6,000 x g for 1min. Remove the supernatant completely and resuspend the cells in 100µl of Buffer RL by pipetting the pellet

Step 2 – Lysis

4. Add 400µl of Buffer RA and 4µl of β-mercaptoethanol to the tube of resuspended cells in Step 1 and shake vigorously to mix. Incubate for 5mins at room temperature
5. Centrifuge at 16,000 x g for 10mins
6. Transfer the supernatant to a new 1.5ml microcentrifuge tube

Step 3 – RNA Binding

6. Add 500µl of 70% ethanol prepared with ddH_2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting)
7. Place a DR Column on a Collection Tube. Add 600µl of the mixture from the previous step to the DR Column
8. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube

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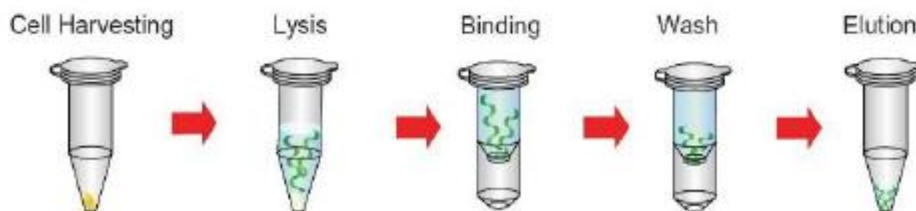
9. Transfer the remaining mixture to the same DR Column
10. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube

Step 4 – Wash

8. Add 400µl of Buffer W1 into the DR Column
9. Centrifuge at 14,000 x g for 30secs
10. Discard the flow-through and place the DR Column back onto the same Collection Tube
11. Add 600µl of Buffer W2 (Ethanol added) into the DR Column
12. Centrifuge at 14,000 x g for 30secs
13. Discard the flow-through and place the DR Column back onto the same Collection Tube
14. Centrifuge at 14,000 x g again for 2mins to remove the residual Buffer W2

Step 5 – RNA Elution

4. To elute RNA, place the DR Column onto a new clean 1.5ml microcentrifuge tube
5. Add 50 - 200µl of Buffer RE to the center of each DR Column, allow it to stand for 2mins
6. Centrifuge at 14,000 x g for 2mins to elute the purified RNA
   *Optional DNase treatments can be followed to remove unwanted DNA residue
   Note: Check buffers for salt precipitation before use. Re-dissolve any precipitate by warming to 37°C



[For Gram-Negative Bacteria Cells]

Step 1 – Sample Cells Harvesting

1. Transfer the cultured Gram-Negative Bacteria cells (up to 10^9) to a sterile 1.5ml microcentrifuge tube
2. Centrifuge at 12,000 x g for 1min. Remove the supernatant completely and resuspend the cells in 200µl of Buffer RO by pipetting the pellet. Incubate for 5mins at room temperature

Step 2 – Lysis

1. Add 300µl of Buffer RA and 3µl of β-mercaptoethanol to the tube of sample lysate in Step 1 and vortex immediately to mix. Incubate for 5mins at room temperature
2. Centrifuge at 16,000 x g for 10mins
3. Transfer the supernatant to a new 1.5ml microcentrifuge tube

Step 3 – RNA Binding

1. Add 500µl of 70% ethanol prepared with ddH_2O (RNase-free and DNase-free) to the

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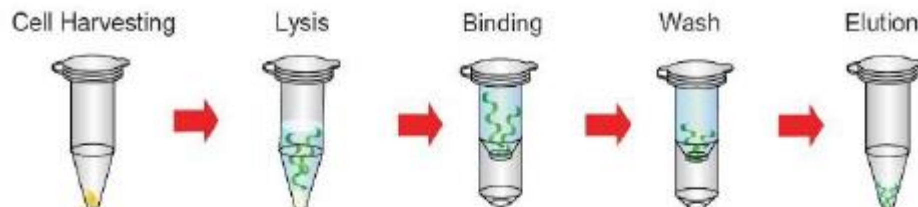
sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting)
2. Place a DR Column on a Collection Tube. Add 600μl of the mixture from the previous step to the DR Column
3. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube
4. Transfer the remaining mixture to the same DR Column
5. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube

Step 4 – Wash

1. Add 400μl of Buffer W1 into the DR Column
2. Centrifuge at 14,000 x g for 30secs
3. Discard the flow-through and place the DR Column back onto the same Collection Tube
4. Add 600μl of Buffer W2 (Ethanol added) into the DR Column
5. Centrifuge at 14,000 x g for 30secs
6. Discard the flow-through and place the DR Column back onto the same Collection Tube
7. Centrifuge at 14,000 x g again for 2mins to remove the residual Buffer W2

Step 5 – RNA Elution

1. To elute RNA, place the DR Column onto a new clean 1.5ml microcentrifuge tube
2. Add 50 - 200μl of Buffer RE to the center of each DR Column, allow it to stand for 2mins
3. Centrifuge at 14,000 x g for 2mins to elute the purified RNA
   *Optional DNase treatments can be followed to remove unwanted DNA residue
   Note: Check buffers for salt precipitation before use. Re-dissolve any precipitate by warming to 37°C



[For Gram-Positive Bacteria Cells]

Step 1 – Sample Cells Harvesting

1. Transfer the cultured Gram-Positive Bacteria cells (up to 10^{9}) to a sterile 1.5ml microcentrifuge tube
2. Centrifuge at 12,000 x g for 1min. Remove the supernatant completely and resuspend the cells in 200μl of Lysozyme Buffer by pipetting the pellet. Incubate for 10mins at room temperature

Step 2 – Lysis

1. Add 300μl of Buffer RA and 3μl of β-mercaptoethanol to the tube of sample lysate in Step

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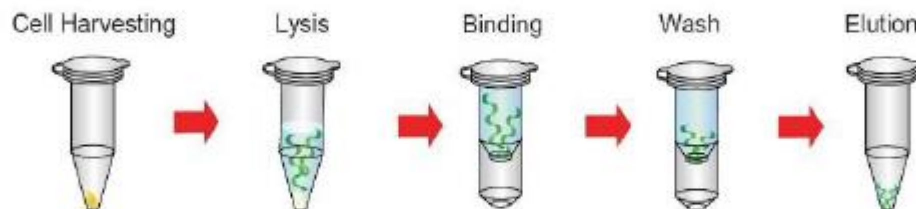
1 and vortex immediately to mix. Incubate for 5mins at room temperature
2. Centrifuge at 16,000 x g for 10mins
3. Transfer the supernatant to a new 1.5ml microcentrifuge tube

Step 3 – RNA Binding
1. Add 500µl of 70% ethanol prepared with ddH_2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting)
2. Place a DR Column on a Collection Tube. Add 600µl of the mixture from the previous step to the DR Column
3. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube
4. Transfer the remaining mixture to the same DR Column
5. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube

Step 4 – Wash
1. Add 400µl of Buffer W1 into the DR Column
2. Centrifuge at 14,000 x g for 30secs
3. Discard the flow-through and place the DR Column back onto the same Collection Tube
4. Add 600µl of Buffer W2 (Ethanol added) into the DR Column
5. Centrifuge at 14,000 x g for 30secs
6. Discard the flow-through and place the DR Column back onto the same Collection Tube
7. Centrifuge at 14,000 x g again for 2mins to remove the residual Buffer W2

Step 5 – RNA Elution
1. To elute RNA, place the DR Column onto a new clean 1.5ml microcentrifuge tube
2. Add 50 - 200µl of Buffer RE to the center of each DR Column, allow it to stand for 2mins
3. Centrifuge at 14,000 x g for 2mins to elute the purified RNA
   *Optional DNase treatments can be followed to remove unwanted DNA residue
   Note: Check buffers for salt precipitation before use. Re-dissolve any precipitate by warming to 37°C



[For Fungus Cells]

Step 1 – Sample Cells Harvesting
1. Transfer the Fungus cells (up to 10^8) to a sterile 1.5ml microcentrifuge tube
2. Centrifuge at 6,000 x g for 5min. Remove the supernatant completely and resuspend the cells in 600µl of Sorbital Buffer by pipetting the pellet

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3. Add 200U of Lyticase or Zymolase. Incubate at the 30°C for 30mins
4. Centrifuge the mixture at 2,000 x g for 10mins to harvest the spheroplast
5. Remove the supernatant completely and resuspend the cells with 200µl of Buffer RO by pipetting the pellet. Incubate for 5 mins at room temperature

Step 2 – Lysis

1. Add 300µl of Buffer RA and 3µl of β-mercaptoethanol to the tube of sample lysate in Step 1 and vortex immediately to mix. Incubate for 5mins at room temperature
2. Centrifuge at 16,000 x g for 10mins
3. Transfer the supernatant to a new 1.5ml microcentrifuge tube

Step 3 – RNA Binding

1. Add 500µl of 70% ethanol prepared with ddH_2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting)
2. Place a DR Column on a Collection Tube. Add 600µl of the mixture from the previous step to the DR Column
3. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube
4. Transfer the remaining mixture to the same DR Column
5. Centrifuge at 14,000 x g for 1min, discard the flow-through and place the DR Column back onto the same Collection Tube

Step 4 – Wash

1. Add 400µl of Buffer W1 into the DR Column
2. Centrifuge at 14,000 x g for 30secs
3. Discard the flow-through and place the DR Column back onto the same Collection Tube
4. Add 600µl of Buffer W2 (Ethanol added) into the DR Column
5. Centrifuge at 14,000 x g for 30secs
6. Discard the flow-through and place the DR Column back onto the same Collection Tube
7. Centrifuge at 14,000 x g again for 2mins to remove the residual Buffer W2

Step 5 – RNA Elution

1. To elute RNA, place the DR Column onto a new clean 1.5ml microcentrifuge tube
2. Add 50 - 200µl of Buffer RE to the center of each DR Column, allow it to stand for 2mins
3. Centrifuge at 14,000 x g for 2mins to elute the purified RNA
   *Optional DNase treatments can be followed to remove unwanted DNA residue
   Note: Check buffers for salt precipitation before use. Re-dissolve any precipitate by warming to 37°C

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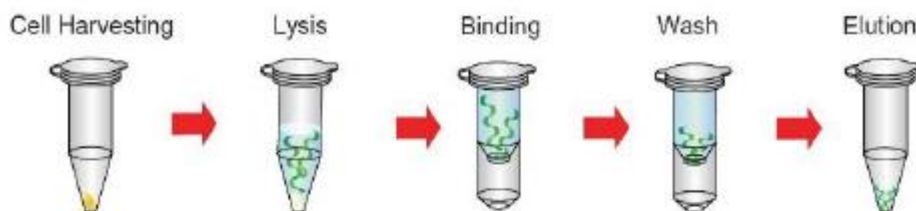

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when using the kit.

Trouble	Cause	Solution
Low yield of RNA	Incomplete lysis and homogenisation	• Use the appropriate method for the lysate preparation based on the amount of the starting materials • Increase the digestion time
	Ethanol not added to Buffer W2	Add 60ml of Absolute Ethanol (96 – 100%) to Buffer W2 and mix well before use
	Incorrect elution conditions	Add 50µl of Buffer RE to the center of each DR Column, let it stand for 2mins, and centrifuge at 14,000 x g for 2mins
RNA got degraded or low integrity	RNases contaminant	• Check buffers for contamination and replace if necessary • Clean workbench before start of experiment • Use new glass and plastic wares, and wear gloves • Use filtered tips and use RNase inhibitor
Inhibition of downstream enzymatic reactions	Presence of residual ethanol in purified RNA	Discard the ethanol of Buffer W2 flow-through from the Collection Tube. Repeat the wash step and centrifuge the spin column at maximum speed for 2-3mins to remove the residual Buffer W2

Caution

- During operation, always wear a lab coat, protective equipment, and latex or vinyl gloves while handling reagents and RNA samples to prevent the RNase contamination
- Check Buffers before use for salt precipitation. Dissolve any precipitate by warming to 37°C and mixing
- Buffers RA and W1 contain irritants. Wear gloves when handling these buffers
- Add 60ml of Absolute Ethanol (96 - 100%) to Buffer W2 and mix well before use
- For Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses

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