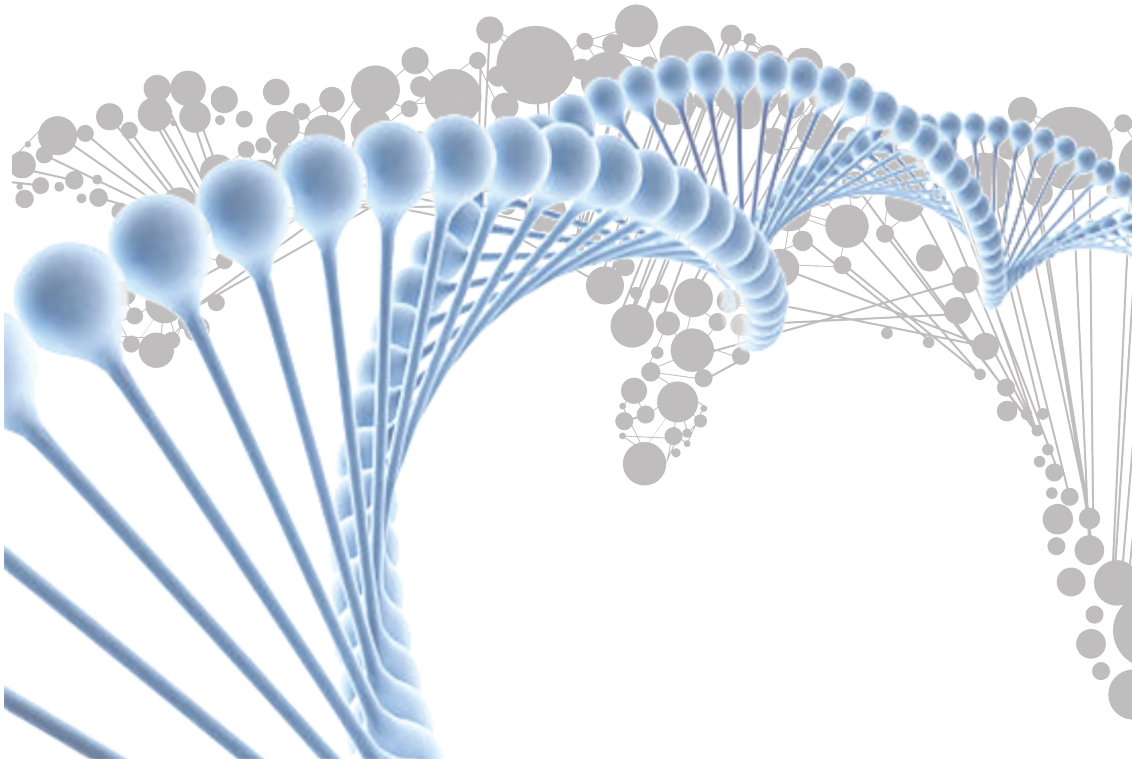
FBRB100

Total RNA Isolation Kit --Blood/ Cell



www.eblbio.com

Blood/ Cell Total RNA Isolation Kit

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: : up to 0.3 ml of whole blood, 10^7 mammalian cells or 10^9 bacterial cells

Introduction:

The EBL Blood/ Cell 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 further allows all RNA bases to bind to the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and pure RNA is eluted with EB Buffer without phenol extraction or alcohol precipitation needs. RNA purified with The 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.

About the kits:

Cat. No.	FBRP-0100
SR Columns	100 pcs
Collection Tubes	100 pcs
BRL Buffer	110 ml
BRA	45 ml
BRO	25 ml
W1 Buffer	45 ml
W2 Buffer	15 ml
EB Buffer	10 ml

Additional requirements:

* **Ethanol (96~100%)**
* **Isopropanol**
* **ß-mercaptoethanol**

***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 $MnCl_2$, 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 $CaCl_2$, 0.1 M Tris-HCl pH 7.5, 35 mM ß-mercaptoethanol).

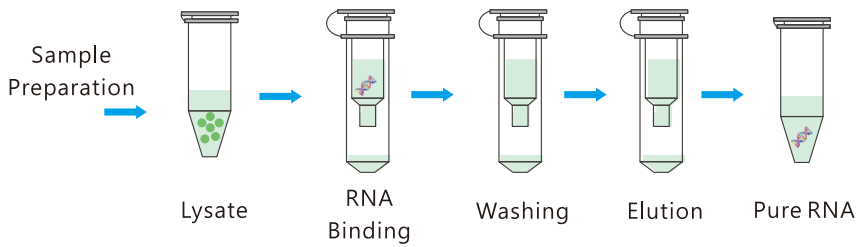
Related product and Ordering information:

CAT NO:	Product	Quantity
MGK-T0100	EBL Genomic DNA Isolation Kit (Tissue)	100 rxn.
MGK-T0300	EBL Genomic DNA Isolation Kit (Tissue)	300 rxn.
MGK-C0100	EBL Genomic DNA Isolation Kit (Cell/ Blood)	100 rxn.
MGK-C0300	EBL Genomic DNA Isolation Kit (Cell/ Blood)	300 rxn.
MGK-P0100	EBL Genomic DNA Isolation Kit (Plant)	100 rxn.
MGK-P0300	EBL Genomic DNA Isolation Kit (Plant)	300 rxn.
MPD-01300	EBL Plasmid Miniprep DNA Kit	300 rxn.
MPD-02025	EBL 200PLUS Plasmid Midiprep DNA Kit	25 rxn
MPD-03020	EBL 800PLUS Plasmid Maxiprep DNA Kit	20 rxn
FBRE100	Blood/ cell Total RNA Isolation kit	100 rxn.
FBRT100	Tissue Total RNA Isolation kit	100 rxn.
FBRP	Plant Total RNA Isolation kit	100 rxn.

NOTE:

1. Wear the gloves while handling reagents and RNA samples to prevent RNase contamination.
2. Add ethanol (96–100%) to Buffer W2 before use (see bottle label for volume).
3. If necessary, re-dissolve any precipitate by warming to 37°C.
4. Buffers BRA and W1 contain irritants. Wear gloves when handling these buffers.

Procedure:



Protocol:

Step 1. Sample Preparation

Fresh Blood

> Collect blood in EDTA-Na2 treated collection tubes (or other anticoagulant mixtures).
> Transfer up to 300 µl of blood to a sterile1.5 ml microcentrifuge tube.
> Add 900 µl of BRL Buffer and mix by inversion.
> Incubate the tube on ice for 10 minutes (invert twice during incubation).
> Centrifuge for 5 minutes at 4,000 x g at 4°C. Remove the supernatant completely and resuspend the cells in 100 µl of BRL Buffer by pipetting the pellet up and down.

Cultured Mammalian Cells

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

Gram-Negative Bacterial Cells

> Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
> Centrifuge at 12,000 x g for 1 minute. Remove the supernatant completely and resuspend the cells in 200 µl of BRO Buffer by pipetting the pellet up and down. Incubate at room temperature for 5 minutes.

Gram-Postive Bacterial Cells

> Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
> Centrifuge at 12,000 x g for 1 minute. Remove the supernatant completely and resuspend the cells in 200 µl of lysozyme Buffer by pipetting the pellet up and down. Incubate at room temperature for 10 minutes.

Fungus Cells

> Transfer fungus cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
> Centrifuge at 6,000 x g for 5 minute. Remove the supernatant completely and resuspend the cells in 600 µl of sorbitol Buffer by pipetting the pellet up and down.
> Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes.
> Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. Remove the supernatant completely and resuspend the cells in 200 µl of BRO Buffer by pipetting the pellet. Incubate at room temperature for 5 minutes.

Step 2. Lysis

Fresh Blood/Mammalian Cells

> Add 400 µl of BRA Buffer and 4 µl of ß-mercaptoethanol to the resuspended cells from Step 1 and shake vigorously. Incubate at room temperature for 5 minutes.
> Centrifuge at 16,000 x g for 10 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Bacterial Cells/Fungus Cells

> Add 300 µl of BRA Buffer and 3 µl of ß-mercaptoethanol to the sample lysate from Step 1 and mix by vortex. Incubate at room temperature for 5 minutes.
> Centrifuge at 16,000 x g for 10 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 Binding

> Add 500 µl of 70% ethanol prepared in ddH2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting).
> Place a SR Column in a Collection Tube. Apply 600µl of the mixture to the SR Column.
> Centrifuge at 14,000 x g for 1 minute. Discard the flow-through and place the SR Column in the same Collection tube. Transfer the remaining mixture to the same SR Column.
> Centrifuge at 14,000 x g for1 minute. Discard the flow-through and place the SR Column in the same Collection tube.

 # optional DNase treatments can be followed on-column Dnase I digestion to remove unwanted DNA residue.

Procedure:

> Add the DNase I incubation mix (80 µl, 4U DNase I in the reaction buffer) directly to the SR column membrane center, and place on the benchtop (20–30°C) for 15 min.
> Following W1 Buffer wash step.

Step 4. Wash

> Add 400 µl of W1 Buffer into the SR Column. Centrifuge at 14,000 x g for 30 seconds.
> Discard the flow-through and place the SR Column back into the same Collection tube.
> Add 600 µl of W2 Buffer (**Ethanol added**) into the SR Column. Centrifuge at 14,000 x g for 30 seconds.
> Discard the flow-through and place the SR Column back into the same Collection tube.
> Centrifuge at 14,000 x g again for 2 minutes to remove residual W2 Buffer.

Step 5. RNA Elution

> Transfer the dried SR Column to a new 1.5 ml microcentrifuge tube.
> Add 50-100 µl EB Buffer to the center of the SR column matrix and let stand for 2-3 minutes.
> Centrifuge for 2 minutes at 14,000 x g to elute the purified RNA.

Troubleshooting

Problem	Comments and suggestions
Degraded RNA / low integrity	
>RNases contaminantion	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	Don`t use more samples than the suggested limit.
>Incorrect elution conditions	Add 100 µl of the EB Buffer to the center of each SR Column, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.
Inhibition of downstream enzymatic reactions	
>Presence of ethanol in the purified RNA	Repeat the wash step: Centrifuge at 14,000 x g again for 2 minutes to remove the residual W2 Buffer.