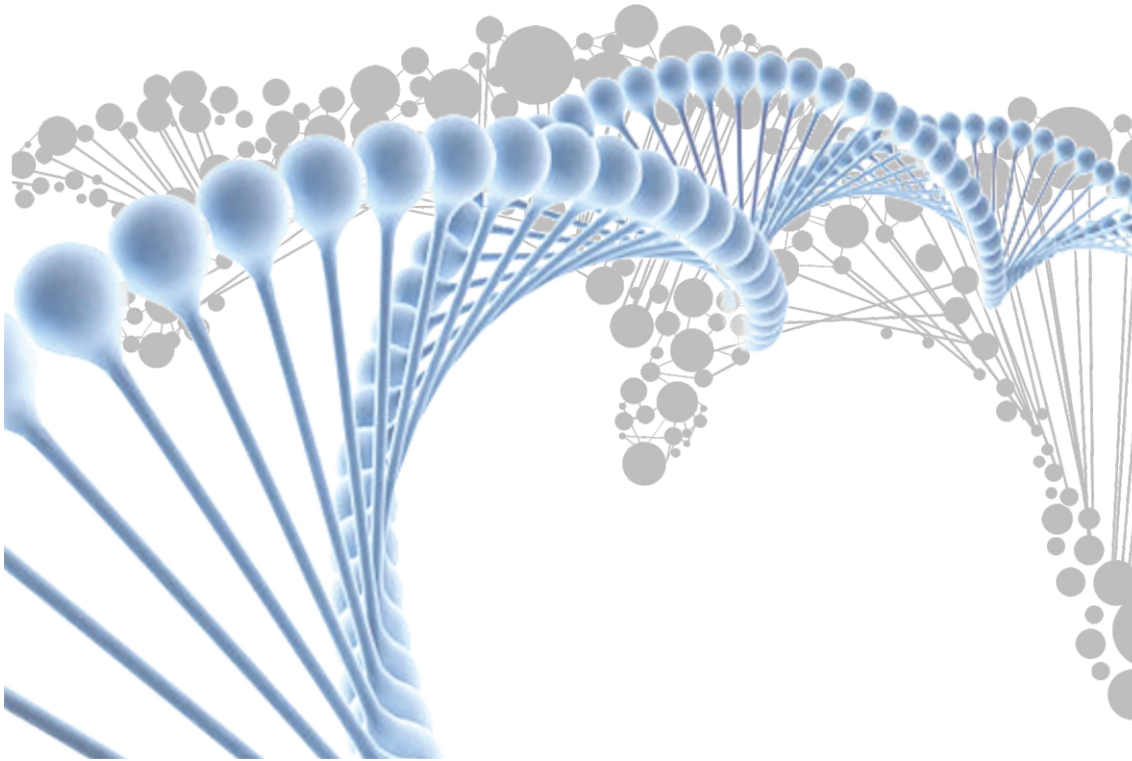
FBRT100

Total RNA Isolation Kit --Tissue



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Tissue Total RNA Isolation Kit

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: up to 30 mg of animal tissue or 25 mg of FFPE tissue

Introduction:

The EBL Total RNA Isolation Kit (Tissue) provides a fast, simple, and cost-effective method for isolation of 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 EB Buffer without phenol extraction or alcohol precipitation. 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.	FBRT-0100
SR Columns	100 pcs
Collection Tubes	100 pcs
RL Buffer	45 ml
W1 Buffer	45 ml
W2 Buffer	15 ml
EB Buffer	10 ml

Additional requirements:

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

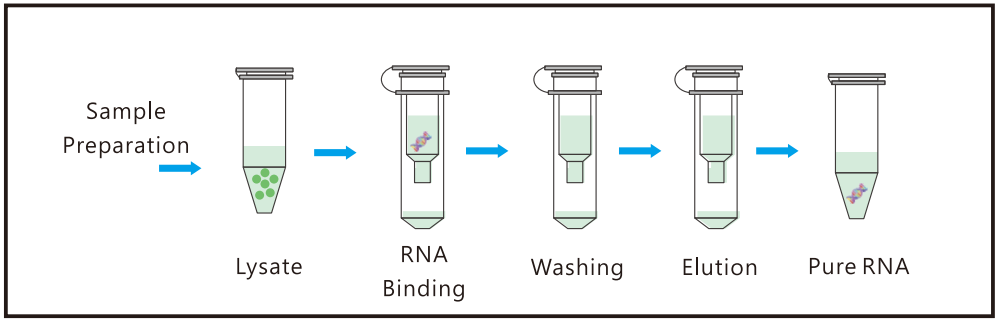
NOTE:

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

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.

Procedure:



Protocol:

Step 1. Sample Preparation

Fresh Tissue

> Cut off up to 30 mg of fresh or frozen animal tissue and grind the sample under liquid nitrogen to a fine powder using a mortar and pestle. (If using frozen animal tissue, the sample MUST have been flash frozen in liquid nitrogen and immediately stored at -70°C until use, to avoid RNA Degradation).

Paraffin-embedded tissue

> Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.
> Add 1 ml of **xylene** to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.
> Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
> Add 1 ml of **absolute ethanol** to wash the sample pellet and mix by inverting.
> Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
> Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
> Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
> Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
> Proceed with the Lysis Step.

Step 2. Lysis

> Add 400 µl of RL Buffer and 4 µl of ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved. Transfer the dissolved sample to a RNase-free 1.5 ml microcentrifuge tube. Incubate at 80°C for 20 minutes. (invert the tube every 10 min.)

> Centrifuge at 16,000 x g for 3 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3. RNA Binding

> Add 400 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.

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 µl EB Buffer to the center of the SR column matrix and let stand for 3 minutes.
> Centrifuge for 2 minutes at 14,000 x g to elute the purified RNA.

 # optional DNase treatments can be followed to remove unwanted DNA residue.

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.