BIO-DOC

Tissue Total RNA Mini Kit

Only for Research.

For isolation RNA from animal cells, animal tissues, bacteria, yeast, paraffin fixed sample, fungi and for RNA clean-up.

Kit Contents:

Cat. No:	BDCK011-004 (4 preps_sample)	BDCK011-50 (50 preps)	BDCK011-100 (100 preps)
RB Buffer	1.5 mL x 2	25 mL	45 mL
Wash Buffer 1	1.5 mL x 2	30 mL	60 mL
Wash Buffer 2 (concentrate)*	1.5 mL	15 mL	35 mL
RNase-free Water	0.5 mL	6 mL	6 mL
Filter Column	4 pcs	50 pcs	100 pcs
RB Mini Column	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
Preparation of Wash Buffer 2 by adding ethanol (96~100%)			
Ethanol volume for Wash Buffer 2 *	6 mL	60 mL	140 mL

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	Please see Table. Sample amount and Yield.
Operation time:	30 ~ 60 minutes
Binding capacity:	up to 100 µg total RNA/ column
Expected yield:	Please see Table. Sample amount and Yield.
Column applicability:	centrifugation and vaccum
Minimum elution volume:	40 µl

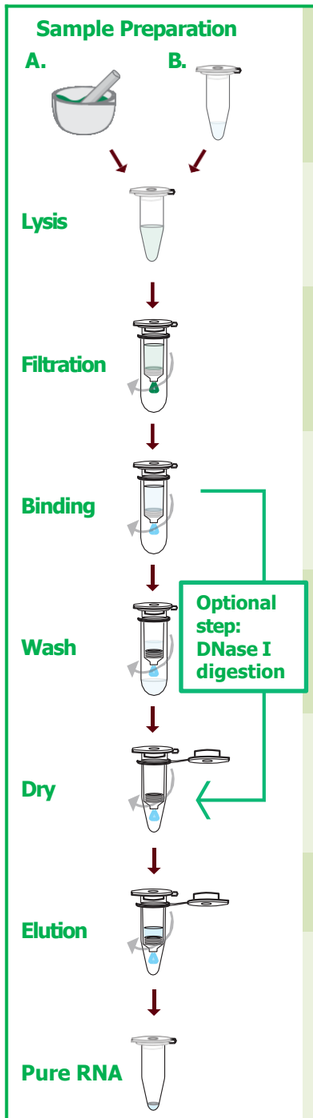
Sample amount and yield:

Sample	Recommended amount of sample used		Yield (µg)
Animal cells (up to 5 x 10^6)	NIH/3T3 HeLa COS-7 LMH	1 x 10^6 cells	10 15 30 12
Animal Tissue (Mouse/rat) (up to 30 mg)	Embryo Heart Brain Kidney Liver Spleen Lung Thymus	10 mg	25 10 10 30 50 35 15 45
Bacteria	*E. coli* *B. subtilis*	1 x 10^9 cells	60 40
Yeast (up to 5 x 10^7)	S. cerevisiae	1 x 10^7 cells	25

Important Notes:

1. Make sure everything is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. **Caution: ß-mercaptoethanol is hazardous to human health. perform the procedures involving RB Buffer Buffer in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to **Wash Buffer 2** when first use.
5. Dilute RNase-free DNase 1 in reaction buffer (1M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25ºC) to final conc. = 0.5 U/µl.

Brief procedure:



A. For Tissue Sample : Homogenization (Grind the sample in liquid nitrogen)

B. For Cultured cells : Centrifuge at 300 x g for 5 min at 4℃.

Add **350 µl of RB Buffer** and **3.5 µl of ß-Mercaptoethanol.**

1. Filtration : Centrifuge at full speed (~18,000 x g) for 2 min.
2. Add 1 volume 70% Ethanol to the supernatant.

Transfer the supernatant to RB Mini Column.
Centrifuge at full speed (~18,000 x g) for 1 min.

1. Add **500 µl of Wash Buffer 1** Centrifuge at full speed (~18,000 x g) for 1 min.
2. Add **750 µl of Wash Buffer 2** Centrifuge at full speed (~18,000 x g) for 1 min.
   Repeat this step for one more washing.

Centrifuge at full speed (~18,000 x g) for an additional 3 min to dry the column.

Add **40 ~ 100 µl of RNase-free ddH_2O** Stand for 1 min.
Centrifuge at full speed (~18,000 x g) for 1 min Store RNA at -70℃.

Optional step:
1. Add **250 µl of Wash Buffer 1** Centrifuge at full speed (~18,000 x g) for 1 min.
2. Add **60 µl of RNase-free DNase 1 solution (0.5 U/µl, not provided).**
   Stand for 15 min.
3. Add **250 µl of Wash Buffer 1** Centrifuge at full speed (~18,000 x g) for 1 min.
4. Add **750 µl of Wash Buffer 2** Centrifuge at full speed (~18,000 x g) for 1 min.
   Repeat this step for one more washing.

General Protocol: For Animal Cells

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Sample preparation	Collect 1 ~5 ×10^6 cells by centrifuge at 300 x g for 5 min at 4℃. Remove all the supernatant.
2 Lysis	Add **350µl of RB Buffer** and **3.5µl of ß-Mercaptoethanol** to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.
3.1 Filtration	1. Place a Filter Column to a Collection Tube and transfer the sample mixture to the Filter Column. Centrifuge at full speed (~18,000 x g) for 2 min. 2. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube(not provided), and measure the volume of the supernatant.
3.2 Ethanol Dilution	Add 1 volume of 70% RNase-free ethanol and mix well by vortexing.
4 RNA Binding	Place a RB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the RB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the RB Mini Column back to the Collection Tube.

5.1 Wash	Add **500 µl of Wash Buffer 1** to the RB Mini Column, centrifugeat at full speed for 1 min. Discard the flow-through and return the RB Mini Column back to the Collection Tube.
	Optional step: DNase I digestion **To eliminate genomic DNA contamination, follow the steps** 1. Add **250 µl of Wash Buffer 1** to the RB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RB Mini Column back to the Collection Tube. 2. Add **60 µl of RNase-free DNase 1 solution** (0.5U/ul, not provided) to the membrane center of the RB Mini Column. Place the column on the benchtop for 15 min. 3. Add **250 µl of Wash Buffer 1** to the RB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RB Mini Column back to the Collection Tube. 4. After DNase 1 treatment, proceed to step 5.2
5.2 Wash	1. Add **750 µl of Wash Buffer 2** to the RB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RB Mini Column back to the Collection Tube. 2. Repeat this step for one more washing.
6 Dry column	Centrifuge the RB Mini Column at full speed for an additional 3 min to dry the RB Mini Column.
7 RNA Elution	1. Place the RB Mini Column to a Elution Tube (provided, 1.5 mL microcentrifuge tube). 2. Add **40 ~100 µl of RNase-free ddH_2O** to the membrane center of the RB Mini Column. Stand the RB Mini Column for 1 min. 3. Centrifuge the RB Mini Column at full speed for 1 min to elute RNA. Store RNA at -70℃.

Special Protocol: The sample preparation For Animal Tissues

Additional requirement	1. Liquid nitrogen 2. Mortar a rotor-stator homogenizer or a 20-G needle syringe 3. ß-Mercaptoethanol 4. 70% RNase-free ethanol
Method 1	1. Weight up to 30 mg of tissue sample. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a new microcentrifuge tube (not provided). **-- Note! Avoid thawing the sample during weighing and grinding.** 2. Add **350 µl of RB Buffer** and **3.5 µl of ß-Mercaptoethanol.** Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times. Incubate at room temperature for 5 min. **-- Important step: In order to release more RNA from the harder samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotot-stator homogenizer.** 3. Follow the General Protocol and start from 3.1 Filtration step.
Method 2	1. Place up to 30 mg of tissue sample to a microcentrifuge tube. **Add 350 µl of RB Buffer** and **3.5 µl of ß-Mercaptoethanol** and use micropestle (not provided) to grind the tissue sample thoroughly. 2. Homogenize the sample by passing lysate through a 20-G needle syringe 10 ~ 20 times. Incubate at room temperature for 5 min. **-- For the tissue samples having low cell amount and hard to disrupt, it is recommended to proceed Method 1 step above.** 3. Follow the General Protocol and start from 3.1 Filtration step.

Special Protocol: The sample preparation For Bacteria

Additional requirement	1. ß-Mercaptoethanol 2. 70% RNase-free ethanol 3. 30℃ water bath or heating block 4. 2 mL screw centrifuge tube 5. Lysozyme reaction solution: (10 mg/mL lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA;1.2% Trition) 6. Acid-washed glass beads, 500 ~ 700 µm
Method	1. Transfer up to 1x10^9 cells well-grown bacterial culture to a 2 mL screw centrifuge tube. **-- Note! Make sure the amount of total RNA harvested from sample do not excess the column's binding capacity (100 µg) when estimate the sample size. Too much sample will make cell lysis incompletely and lead to lower RNA yield and purity. If RNA amount is hard to determin on some species, using ≤5x 10 cells as the starting sample size.** 2. Descend the bacterial cells by centrifuge at full speed (~18,000 x g) for 2 min at 4℃. Remove all the supernatant. 3. Add **100 µl of lysozyme reaction solution.** Pipet up and down to resuspend the cell pellet and incubate at 37℃ for 10 min. 4. Add **350 µl of RB Buffer** and **3.5 µl of ß-Mercaptoethanol.** 5. Add 250 mg of acid-washed glass beads (500 ~700 nm) and vortex vigorously for 5 min to disrupt the cells. 6. Centrifuge at full speed (~18,000 x g) for 2 min to spin down insoluble material. Transfer the supernatant to a microcentrifge tube (not provided) and measure the volume of the clear lysate. **-- Note : Avoid pipetting any debris and pellet in the Collection Tube.** 7. Follow General Protocol and start from 3.2 Ethanol Dilution step.

Special Protocol: The sample preparation For Yeast

Additional requirement	1. ß-Mercaptoethanol 2. 70% RNase-free ethanol 3. Method 1: 2 mL screw centrifuge tube Acid-washed glass beads, 500 ~ 700 µm 4. Method 2: Lyticase or zymolase Sorbitol buffer (1M sorbitol; 100 mM EDTA; 0.1% ß-ME) 30℃ water bath or heating block
Method 1	1. Collect up to **5 x 10^7** of yeast culture by centrifuge at **5,000 x g** for **10 min** at **4℃**. Remove all the supernatant. 2. Add **350 µl of RB Buffer** and **3.5 µl of ß-Mercaptoethanol** to the pellet and vortex vigorously to resuspend the cells completely. 3. Transfer the sample mixture to a 2 mL screw centrifuge tube and add 250 mg of acid-washed glass beads (500 ~700 µm) and vortex vigorously for 15 min to disrupt the cells. 4. Follow General Protocol and start from 3.2 Ethanol Dilution step.
Method 2	1. Collect up to 5 x 10^7 of yeast culture by centrifuge at 5,000 x g for 10 min at 4℃. Remove all the supernatant. 2. Resuspend the cell pellet in **600 µl sorbitol buffer** (1M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided). 3. Add 200 U zymolase or lyticase and incubate at 30℃ for 30 min. **--Note : Prepare sorbitol buffer just before use.** 4. Centrifuge at 300 x g for 5 min to pellte the spheroplasts. Remove all the supernatant. 5. Add **350 µl of RB Buffer** and **3.5 µl of ß-Mercaptoethanol** to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incbuate sample mixture at room temperature for 5 min. 6. Follow General Protocol and start from 3.2 Ethanol Dilution step.

Special Protocol: The sample preparation For Paraffin-embedded tissue

Additional requirement	1. xylene & ethanol (96~100%) 2. liquid nitrogen 3. Mortar a rotor-stator homogenizer or a 20-G needle syringe 4. ß-Mercaptoethanol 5. 70% RNase-free ethanol
Method	1. Transfer up to 15 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided). **-- Remove the extra paraffin to minimize the size of the sample slice.** 2. Add **0.5 mL xylene**, mix well and incubate at room temperature for 10 min. 3. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting. 4. Add **0.25 mL xylene**, mix well and incubate at room temperature for 3 min. 5.Centrifuge at full speed for 3 min. Remove the supernatant by pipetting. 6 .Repeat step 4 and step 5 7. Add **0.3 mL ethanol (96- 100 %)**to the deparaffined tissue, mix gently by vortexing. Incubate at room temperature for 3 min. 8. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting. 9. Repeat step 7 and step 8. 10. Follow **Animal tissue Protocol** starting from step 1 for sample disruption.

Special Protocol: The sample preparation For RNA Clean up

Additional equipment	xylene & ethanol (96~100%)
Method	1. Trandfer 100 µl of RNA sample to a microcentrifuge tube (not provided). **-- If the RNA sample is less than 100 µl, add RNase-free water to make the sample volume to 100 µl.** **2. Add 300 µl of RB Buffer** and **300 µl of RNase-free** ethanol (96~100 %) and mix well by vortexing. 3. Place a RB Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the RB Mini Column. Centrifuge at full speed for 1 min and discard the flow-through and return the RB Mini Column back to the Collection Tube. 4. Follow Animal Cells Protocol starting from step 5.1

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