ALPHAGEN® BIOTECH LTD.

α^+ Solution™ Blood / Cultured Cell Total RNA Mini Kit

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

Kit Contents:

Cat. No:	APBRK 004 (4 preps_sample)	APBRK 050 (50 preps)	APBRK 100 (100 preps)
RL Buffer	15 ml	120 ml	240 ml
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
User Manual	1	1	1
Preparation of Wash Buffer 2 by adding ethanol (96 ~ 100%)			
Ethanol volume for Wash Buffer 2 *	6 ml	60 ml	140 ml

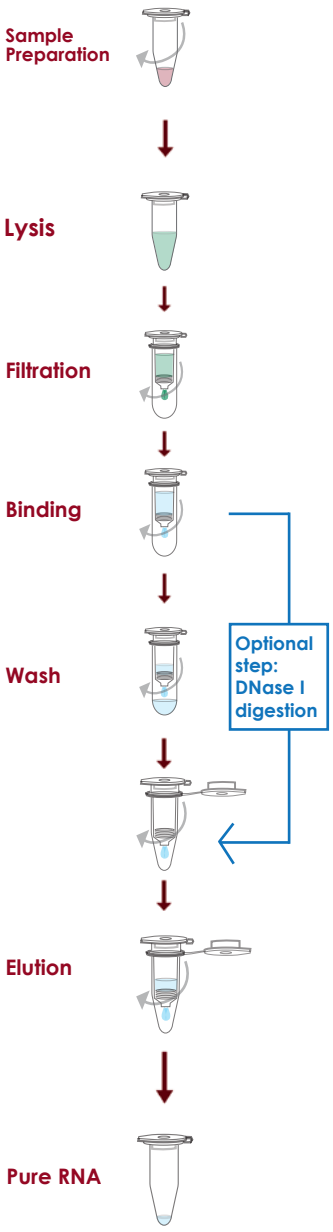
Specification:

Principle:	mini spin column (silica matrix)
Sample size:	300 µl
Operation time:	30 ~ 60 minutes
Binding capacity:	up to 100 µg total RNA/ column
Expected yield:	1 µg
Column applicability:	centrifugation and vaccum
Minimum elution volume:	40 µl

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 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 25°C) to final conc. = 0.5 U/µl.

Brief procedure:



1. Add 200 ~ 300 µl whole blood and 5 volume of RL Buffer
2. Incubate on ice for 10 min.
3. Centrifuge for 1 min at 4,500 rpm (600 x g) and discard the supernatant completely.
4. Add 600 µl of RL Buffer.
5. Centrifuge for 1 min at 4,500 rpm (600 x g) and discard the supernatant completely.

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.

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$_2$O** 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 (0.5U/ul, 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

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Sample preparation	1. Add 200 ~ 300 µl of anticoagulant-preserved fresh human whole blood to a microcentrifuge tube (1.5 ml or 2.0 ml tube) (not rovided). If the sample volume is more than 200 ul, use a 2.0 ml tube as the sample container. **-- Note! Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.** 2. Add 5 volume of RL Buffer to the sample and mix well by inversion. 3. Incubate on ice for 10 min. Mix by vortexing briefly 2 times during incubation. 4. Centrifuge for 1 min at 4,500 rpm (600 x g) to form a cell pellet and discard the supernatant completely. 5. Add **600 µl of RL Buffer** to resuspend the cell pellet by vortexing briefly. 6. Centrifuge for 1min at 4,500 rpm (600 x g) to form a cell pellet again and discard the supernatant completely.
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℃ .

Scan to discover !

https://www.alphagen.bio

Scan to ALPHAGEN troubleshooting