FISHER MOLECULAR BIOLOGY

BLOOD/TISSUES TOTAL RNA EXTRACTION MINI KIT RE-003 & RE-004

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

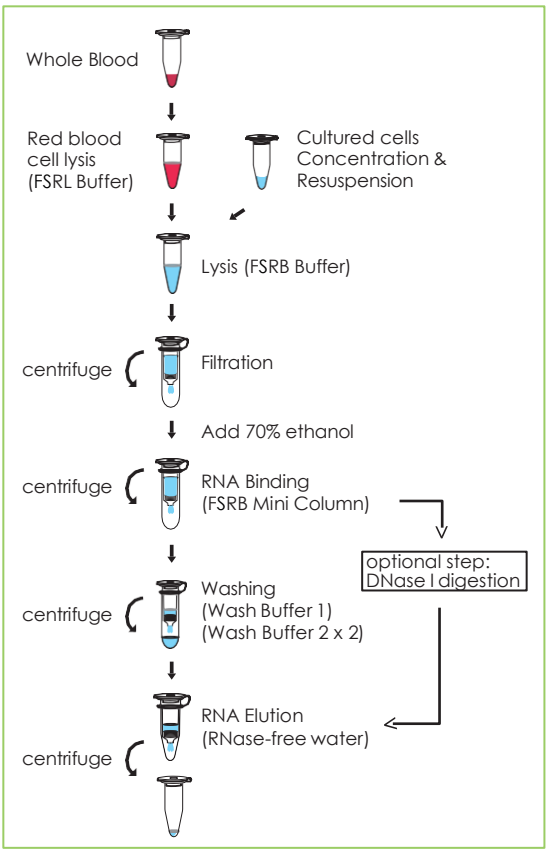
KIT Components	RE-003s SAMPLE	RE-003 50 PREPS	RE-004 100 PREPS	RE-004B 300 PREPS
FSRL Buffer	15 ml	120 ml	240 ml	240 ml x 2
FSRB Buffer	1,5 ml x 2	25 ml	45 ml	130 ml
Wash Buffer 1	1,5 ml x 2	30 ml	60 ml	3 x 60 ml
Wash Buffer 2 (concentrate) [a]	1.5 ml	15 ml	35 ml	3 x 35 ml
RNase-free Water	0.5 ml	6 ml	6 ml	3 x 6 ml
Filter Column	4 pcs	50 pcs	100 pcs	300 pcs
FSRB Mini Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
Micropestles	4 pcs	50 pcs	100 pcs	300 pcs
DNAse I 0,5U/ul		1 mg	1 mg	1 mg
Preparation of Wash Buffer 2 by adding ethanol (96 ~ 100%)				
Ethanol volume for (concentrate) [a]	6 ml	60 ml	140 ml	3x 140 ml

Specifications:

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

Sample	Recommended amount of sample used		Yield (µg)
Human whole blood (up to 300 µl)	300 µl		1
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 10^9cells	60 40
Yeast (up to 5 x 10^7)	S. cerevisiae	1 x 10^7cells	25

Brief procedure:



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: Beta-mercaptoethanol (Beta-Me) is hazardous to human health. perform the procedures involving Beta-Me in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 when first use.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
6. Prepare RNase-free DNase 1 reaction buffer (1M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C) and make the final concentration of DNase I to 0.5 U/µl. (we recommend to use DNAse I)
7. Dilute 1 mg of DNase I in 1 ml of DNase Buffer supplied with the kit, to obtain a final concentration of 0.5U / ul

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Protocol: Isolation of Total RNA from Human Whole Blood

Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol and 70% RNase-free ethanol

1. Red blood cells lysis

1-1. Add 200 ~ 300 µl of anticoagulant-preserved fresh human whole blood to a microcentrifuge tube (1.5 ml or 2.0 ml tube) (not provided). 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.

1-2. Mix 5 volume of FSRL Buffer with 1 volume of the sample and mix well by inversion.

1-3. Incubate on ice for 10 min. Vortex briefly 2 times during incubation.

1-4. Centrifuge for 1 min at 4,500 rpm to form a cell pellet and discard the supernatant completely.

1-5. Add 600 µl of FSRL Buffer to resuspend the cell pellet by briefly vortexing.

1-6. Centrifuge for 1min at 4,500 rpm to form a cell pellet again and discard the supernatant completely.

2-Add 350 µl of FSRB Buffer and 3.5 µl of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.

-- Note: If the clump is still visible after vortex, pipet sample mixture up and down to break down the clump.

3-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.

4-Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided), and measure the volume of the supernatant.

-- Note: Avoid to pipet any debris and pellet when transfering the supernatant.

5-Add 1 volume of 70 % RNase-free ethanol and mix well by vortexing.

6-Place a FSRB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FSRB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

7-Optional step: DNase I digestion To eliminate genomic DNA contamination, follow the steps from 7a. Otherwise, proceed to step 8 directly.

Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

Add 60 µl of RNase-free DNase 1 solution (0.5U/ul, not provided) to the membrane center of the FSRB Mini Column. Place the column on the benchtop for 15 min.

Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at ful speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

After DNase 1 treatment, proceed to step 9.

8-Add 500 µl of Wash Buffer 1 to the FSRB Mini Column, centrifugeat at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

9-Add 750 µl of Wash Buffer 2 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube. **-- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.**

10- Repeat step 9 for one more washing.

11- Centrifuge the FSRB Mini Column at full speed for an additional 3 min to dry the FSRB Mini Column. **-- Important Step!This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.**

12- Place the FSRB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).

13- Add 40 ~ 100 µl of RNase-free ddH2O to the membrane center of the FSRB Mini Column. Stand the FSRB Mini Column for 1 min.

-- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.

-- Important : Do not elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will lower the RNA yield.

14- Centrifuge the FSRB Mini Column at full speed for 1 min to elute RNA.

15- Store RNA at -70°C.

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Protocol: Isolation of Total RNA from Animal Cells

Read Important Notes Before Starting Following Steps.

Additional requirment:

- ß-Mercaptoethanol
- 70% RNase-free ethanol

1. Collect 1 - 5 ×10^6 cells by centrifuge at 300 x g for 5 min at 4 °C. Remove all the supernatant.
   -- Note! Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yiel and purity.

2. Add 350 ul of FSRB Buffer and 3.5 ul of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.
   -- Note: If the clump is still visible after vortex, pipet sample mixture up and down to break down the clump.
3. Follow Human Whole Blood Protocol starting from step 3.

Protocol: Isolation of Total RNA from Animal Tissues (fresh & frozen)

Read Important Notes Before Starting Following Steps.

Additional equipment:

- liquid nitrogen & mortar
- a rotor-stator homogenizer or a 20-G needle syringe
- ß-Mercaptoethanol
- 70% RNase-free ethanol

1a.**(For Fresh sample):** Cut up to 30 mg of tissue sample. Grind the tissue sample completely in liquid nitrogen. Transfer the powder to a new microcentrifuge tube(not provided). Or you can place tissue sample into a microcentrifuge tube and use provided micropestle to grind the tissue sample few times and break it into small pieces.

1.b (For Frozen sample) Weight up to 30 mg tissue sample and grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube (not provided).

2. Add 350 ul of FSRB Buffer and 3.5 ul 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 Human Whole Blood Protocol starting from step 3.

Protocol: Isolation of Total RNA from Paraffin-embedded tissues

Read Important Notes Before Starting Following Steps.

Additional equipment:

- xylene & ethanol (96-100%)
- liquid nitrogen & mortar
- a rotor-stator homogenizer or a 20-G needle syringe
- ß-Mercaptoethanol
- 70% RNase-free ethanol

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 then follow Human Whole Blood protocol starting from step 3.

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Protocol: RNA Clean-Up

Read Important Notes Before Starting Following Steps.

Additional equipment: xylene & ethanol (96-100%)

1. Transfer 100 Ul of RNA sample to a microcentrifuge tube (not provided).
-- If the RNA sample is less than 100 ul, add RNase-free water to make the sample volume to 100 ul.

2. Add 300 ul of FSRB Buffer and 300 ul of RNase-free ethanol (96-100 %) and mix well by vortexing.
3. Ulace a FSRB Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the FSRB Mini Column. Centrifuge at full speed for 1 min and discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
4. Follow Human Whole Blood Protocol starting from step 8.

Protocol: Isolation of Total RNA from Bacteria

Read Important Notes Before Starting Following Steps.

Additional requirement:

- ß-Mercaptoethanol
- 70% RNase-free ethanol
- 30 °C water bath or heating block
- 2 ml screw centrifuge tube
- Lysozyme reaction solution: (10mg/ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA;1.2% Trition)
- Acid-washed glass beads, 500 - 700 um

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 incomuletely and lead to lower RNA yield and purity. If RNA amount is hard to determin on some species, using ≤ 5 x 10^{8} 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 °C. Remove all the supernatant.
3. Add 100 Ul of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate at 37°C for 10 min.
4. Add 350 Ul of FSRB Buffer and 3.5 Ul 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 Human Whole Blood Protocol starting from step 5.

Protocol: Isolation of Total RNA from Yeast

Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol
70% RNase-free ethanol
Enzymatic disruption: Lyticase or zymolase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
30 °C water bath or heating block
Mechanical disruption: 2 ml screw centrifuge tube
Acid-washed glass beads, 500 - 700 um

1. Collect up to 5 x 10^{7} of yeast culture by centrifuge at 5,000 x g for 10 min at 4 °C. Remove all the supernatant.

2A.Enzymtic disruption:
2A-1: Resuspend the cell pellet in 600 Ul sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolase or lyticase and incubate at 30 °C for 30 min.
--**Note! Prepare sorbitol buffer just before use.**
2A-2. Centrifuge at 300 x g for 5 min to pellte the spheroplasts. Remove all the supernatant.
2A-3. Add 350 Ul of FSRB Buffer and 3.5 Ul of ß-Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incbuate sample mixture at room temperature for 5 min

2B. Mechanical disruption:
2B-1. Add 350 Ul of FSRB Buffer and 3.5 Ul of ß-Mercaptoethanol to the pellet and vortex vigorously to resuspend the cells completely.
2B-2. Transfer the sample mixture to a 2 ml screw centrifuge tube and add 250 mg of acid-washed glass beads (500 - 700) and vortex vigorously for 15 min to disrupt the cells.

- Follow Human Whole Blood Protocol starting from step 5

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