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From Blood, Tissue and Plant

Research Use Only | Store at Room Temp.

Product Code: 10119

Size:50 preparations

Description

AddPrepTotal RNA Extraction Kit offer simple, rapid and cost-effective method for isolating Total RNA from whole blood, animal tissue, mammalian cell, biological fluids and plant including any fruits.The yield of RNA extracted from 25~100 mg of tissue, 50~100 mg of plant (including fruits) and 200 µl of whole blood is 5 ~ 30µg. The total RNA extraction is based on a specific RNA binding spin column method with special buffers and an on-column DNase I treatmentfor removing traces of DNA during RNA extraction. The extracted total RNA can be adjusted in variable applications, such as molecular biology experiments including RT-PCR, blotting and so on.

Kit Components

Solution & Material	Size	Usable Solution & Material	
		Blood	Tissue, Cell, Hair root, Biological fluid, Plant, Fruits
Spin column 1 (White ring)	50 ea	●	●
Spin column 2 (Green ring)	50 ea	●	●
Blood Lysis	75 ml	●	
Lysis	25 ml		●
Binding	50 ml	●	●
Washing 1	30 ml **(Add Ethanol 22.5 ml)**	●	●
Washing 2	10 ml **(Add Ethanol 40 ml)**	●	●
Elution	25 ml	●	●
DNase I Reaction Buffer	1 ml X 3 tubes	●	●
Proteinase K (20 mg/ml)	1.2 ml X 1 tubes	●	●
DNase I (1 U/µl)	0.5 ml X 1 tube	●	●

Storage and Stability

AddPrepTotal RNA Extraction Kit is stable for 3 years when stored in a constant temperature 15 ~ 35℃.

Before You Begin

1. Add ethanol to Washing 1 and Washing 2 Solution before use.
2. Check Lysis, Binding and Washing 1 Solution for any precipitation, and any precipitant can be dissolved by warming at 50℃.

Extraction Protocol for Whole Blood

1. Add 200 µl of whole blood and 1 ml of Blood Lysis Buffer to a 1.5 ml micro-centrifuge tube (Not provided) and mix by vortexing:
If the sample volume is less than 200 µl, add the appropriate volume of PBS.

2. Incubate at less than 5℃ the 1.5 ml tube included whole blood and Blood Lysis Buffer:
Mix welloccasionally during incubation to disperse the sample

3. Centrifuge at 13,000 rpm for 1 minute and discard the supernatant for removing the red blood cell.

4. Add 400 µl of Blood Lysis Buffer to the tube with white blood cell and mix well by pulse-vortexing.

5. Centrifuge at 13,000 rpm for 1 minute and discard the supernatant carefully.

6. Add 400 µl of Lysis Solution, 4 µl ß-mercaptoethanol and 20 µl Proteinase K solution (20 mg/ml) and resuspend the cell pellet by pipetting or vortexing.

7. Incubate at room temperature (20~30℃)for 10 minutes.

8. Spin down the tube briefly to remove any drops from inside of sample tube lid.

9. Carefully transfer the lysate into the upper reservoir of the spin column 1 (White ring) with 2.0ml collection tube without wetting the rim.

10. Centrifuge at 13,000 rpm for 3 minutes: Save the flow-through

11. Add 400 µl of 70% ethanol to the sample flow-through in a collection tube and mix well by pulse-vortexing for 10 sec: After this step, briefly spin down to get the drops clinging under the lid.(Continued to back page)

12. Carefully transfer the lysate (normally 800 µl) into the upper reservoir of the spin column 2 (Green ring) with 2.0ml collection tube without wetting the rim.
13. Centrifuge at 13,000 rpm for 10sec.: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
14. Add 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 10sec.
15. Centrifuge at 13,000 rpm for 10sec.: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
16. In a RNase-free tube, add 10 µl of DNase (1 U/µl), 40µl of DNase I Reaction Buffer and mix. Add the mixture directly on column matrix.
17. Incubate at room temperature (20~30℃)for 15 minutes.
18. Add 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 minute: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
10. Add 700 µl of Washing 2 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 minute: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
11. Dry the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.
12. Transfer the spin column to the new 1.5 ml micro-centrifuge tube (Not provided).
13. Add 50 ~ 100 µl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 minute.
14. Elute the total RNA by centrifugation at 13,000 rpm for 1 minute.
(The extracted RNA can be used immediately or store at -70℃)

Extraction Protocol for Cell, Tissue, Biological fluid, Plant and Fruits

1. Prepare a fresh or frozen sample for RNA extraction
Cut off 25~100 mg of tissue, 50~100 mg of plant (including fruits), up to 5~10 hair roots and up to 200 µl of biological fluid.
Grind the plant sample to a fine powder in liquid nitrogen using a mortar and pestle.
2. Add 400 µl of Lysis Buffer, 4 µl ß-mercaptoethanoland 20 µl Proteinase K solution (20 mg/ml)to a 1.5 ml micro-centrifuge tube with sample and mix by vortexing:
3. Incubate at 56℃for 10 minutes and centrifuge at 13,000 rpm for 3 minutes.
4. Carefully transfer thesupernatant into the upper reservoir of the spin column 1 (White ring) with 2.0ml collection tube.
5. Centrifuge at 13,000 rpm for 30sec.: Save the flow-through
6. Add 400 µl of Binding Bufferto the sample flow-through in a collection tube and mix well by pulse-vortexing for 10 sec.
7. Centrifuge at 13,000 rpm for 1 minute.
8. Transfer 500~600 µl supernatant to a new 1.5 ml micro-centrifuge tube, and then add same volume of Binding Buffer and 200 µl absolute ethanol and mix well.
9. Transfer 600 µl of lysateinto the upper reservoir of the spin column 2 (Green ring) with 2.0ml collection tube without wetting the rim.
10. Centrifuge at 13,000 rpm for 10sec.: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
11. Repeat step 10 and 11 using remained lysate.
12. Add 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 10sec.
13. Centrifuge at 13,000 rpm for 10sec.: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
14. In a RNase-free tube, add 10 µl of DNase (1 U/µl), 40µl of DNase I Reaction Buffer and mix. Add the mixture directly on column matrix.
15. Incubate at room temperature (20~30℃)for 15 minutes.
16. Add 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 minute: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
17. Add 700 µl of Washing 2 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 minute: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
18. Dry the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.
19. Transfer the spin column to the new 1.5 ml micro-centrifuge tube (Not provided).
20. Add 50 ~ 100 µl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 minute.
21. Elute the total RNA by centrifugation at 13,000 rpm for 1 minute.
(The extracted RNA can be used immediately or store at -70℃)