HiPure FFPE RNA Plus Kit

Introduction

HiPure FFPE RNA Plus Kit supplies a simple and rapid RNA extraction for Formalin-fixed, paraffin-embedded (FFPE) tissue and sections samples. This kit is based on silica gel column purification technology, no phenol-chloroform extraction or alcohol precipitation. The whole extraction only takes 30 minutes (not including digestion time). RNA can be directly used for downstream applications such as RT-PCR, Northern blot, vitro translation and other experiments.

Principle

This product is based on silica column purification. Remove paraffin by Buffer DPS. Sample lysis with proteinase K digestion requires only 15 minutes. After lysis, samples are incubated at 80℃ for 15 minutes. Transfer to an adsorption column and RNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, RNA was finally eluted with low-salt buffer.

Kit Contents

Product	R414402	R414403	Contents
Purification times	50	250	—
HiPure RNA Micro Columns	50	250	Silica Column
2ml Collection Tubes	50	2 x 125	PP Column
Buffer DPS	60 ml	250 ml	Deparaffinization Buffer
Buffer FRL	15 ml	60 ml	Tris/EDTA/SDS
Buffer RLC	15 ml	60 ml	Guanidine Salt
Buffer RWC*	10 ml	50 ml	Guanidine Salt
Buffer RW2*	20 ml	2 x 50 ml	Tris/EDTA
DNase I	600 µl	5 x 600 µl	DNase I
DNase Booster Buffer	1.5 ml	6 ml	Tris/CaCl2
Protease Dissolve Buffer	1.8 ml	10 ml	Glycerol/Tris/CaCl2
Proteinase K	24 mg	120 mg	Protease
RNase Free Water	10 ml	20 ml	DEPC-Treated Water

Storage and Stability

Proteinase K should be stored at 2–8°C upon arrival. DNase I should be stored at -20°C. However, short-term storage (DNase I up to 1 weeks, Proteinase K up to 8 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

Materials and Equipment to be Supplied by User

- Add 20ml (50 Preps) or 100ml (250 Preps) absolute ethanol to the bottle of Buffer RWC and store at room temperature.
- Add 80ml (50 Preps) or 2 x 200ml (250 Preps) absolute ethanol to the bottle of Buffer RW2 and store at room temperature.
- Add 1.2ml (50 Preps) or 6ml (250 Preps) ml Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Absolute ethanol (96-100%)

Protocol

1. Using a scalpel, trim excess paraffin off the sample block. Cut up to 8 sections 5~20μm thick and immediately place the sections in a 1.5 or 2 ml microcentrifuge tube.

   If the sample surface has been exposed to air, discard the first 2–3 sections,

2. **Add 0.6ml Buffer DPS into sample and vortex for 5 seconds to mix thoroughly**.centrifuge briefly to bring the sample to the bottom of the tube.

3. Incubate at 56°C for 3~5 min and vortex for 5 seconds to dissolve paraffin.

   If too little Buffer DPS is used or if too much paraffin is carried over with the sample, the Buffer DPS may become waxy or solid after cooling. If this occurs, add additional Buffer DPS and repeat the 56°C incubation.

4. Centrifuge at 14,000 x g for 2 minutes. Aspirate and discard the supernatant carefully, do not disturb the pellet.

5. **Add 200μl Buffer FRL and 20μl Proteinase K to the sample and vortex.** Incubate at 55℃ for 15 minutes, then incubate at 80℃ for 15 minutes.

Incubation at 80℃ can reverse the nucleic acids modified by formaldehyde. Prolonged incubation time will cause degradation of RNA.

6. Incbuate on ice for 5 min and centrifuge at 15,000 x g for 5min.Transfer the supernature into a new tube.

7. Add 20μl DNase Booster to the sample. Mix well and incubate on ice for 2 min.

8. Add 10 μl DNase I to the sample and mix well. Incubate at room temperature for 15 min.

9. Add 200 μl Buffer RLC to the sample and mix well.

10. **Add 600 μl of absolute ethanol to the sample and mix well.**

11. **Insert a HiPure RNA Micro Column in a 2ml Collection Tube**.

12. **Transfer half of the mixture to the column,** centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and insert the column to the collection tube.

13. Transfer the remaining mixture to the column, centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and insert the column to the collection tube.

14. **Add 500μl Buffer RWC to the column.** Centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and reuse collection tube.

15. **Add 500μl Buffer RW2 to the column.** Centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and reuse collection tube.

16. **Add 500μl Buffer RW2 to the column.** Centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and reuse collection tube.

17. Centrifuge the empty Column at 8,000 x g for 3 minutes to dry the column matrix.

18. **Transfer the Column to a clean 1.5ml centrifuge tube. Add 15~50μl RNase free water directly to the center of the column membrane.** Stay at room temperature for 2 minutes. Centrifuge at 13,000 x g for 1 minute.

19. Discard the column. Store RNA at -80℃.

Troubleshooting Guide

Low RNA yield

- The starting sample is too much: refer to Protocol
- Sections are too thick: Paraffin tissue sections are too thick, not more than 10 μm.
- Paraffin residue: Xylene is not completely removed from paraffin.

RNA degradation

- The RNA in the sample has been degraded: the paraffin-embedded tissue RNA will be degraded during the process of fixation, embedding and storage.
- RNase contamination: RNase contamination is introduced during extraction.

Downstream experimental results are not ideal

- Salt contamination: After adding Buffer RW2, let it stand for 5 minutes before centrifugation.
- Ethanol contamination: ensure that the empty column centrifugation speed is higher than or equal to 12,000xg, and the centrifugation time is 2 minutes.

Membrane material shedding

- The silica gel membrane may come off during the centrifugation process. The silica membrane that falls off the plasmid is insoluble and may be removed by centrifugation at 12,000xg for 2 minutes.