

HiPure FFPE RNA Kit

Introduction

HiPure FFPE RNA 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°C 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	IVD4144-20	IVD4144	Contents
Purification times	20	50	—
HiPure RNA Micro Columns	20	50	Silica Column
2ml Collection Tubes	20	50	PP Column
Buffer DPS	25 ml	60 ml	Deparaffinization Buffer
Buffer FRL	6 ml	15 ml	Tris/EDTA/SDS
Buffer RLC	6 ml	15 ml	Guanidine Salt
Buffer RVC*	5 ml	10 ml	Guanidine Salt
Buffer RW2*	10 ml	20 ml	Tris/EDTA
DNase I	600 µl	600 µl	DNase I
DNase Booster Buffer	1.5 ml	1.5 ml	Tris/CaCl ₂
Protease Dissolve Buffer	1.8 ml	1.8 ml	Glycerol/Tris/CaCl ₂
Proteinase K	12 mg	24 mg	Protease
RNase Free Water	5 ml	10 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 (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 10ml (20 Preps), or 20ml (50 Preps) absolute ethanol to the bottle of Buffer RWC and store at room temperature.
- Add 40ml (20 Preps), or 80ml (50 Preps) absolute ethanol to the bottle of Buffer RW2 and store at room temperature.
- Add 0.6ml (20 Preps), or 1.2ml (50 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8°C 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°C for 15 minutes, then incubate at 80°C for 15 minutes.

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

6. Incubate on ice for 5 min and centrifuge at 15,000 x g for 5 min. Transfer the supernatant into a new tube.
7. Add 20 µl 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 500 µl Buffer RWC to the column.** Centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and reuse collection tube.
15. **Add 500 µl Buffer RW2 to the column.** Centrifuge at 8,000 x g for 30~60 seconds. Discard the filtrate and reuse collection tube.
16. **Add 500 µl 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 15~50 µl 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 -20 °C or -80 °C..

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 μ m.
- 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.