HiPure FFPE DNA Kit

Introduction

HiPure FFPE DNA Kit supplies a simple and rapid DNA 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 20 minutes (not including digestion time). DNA can be directly used for downstream applications such as PCR, Southern Blot and viral DNA detection, ect.

Kit Contents

Product	IVD3126-20	IVD3126	Contents
Purification times	20 Preps	100 Preps	—
HiPure DNA Mini Columns I	20	100	Silica Column
2ml Collection Tubes	20	100	PP Column
Buffer DPS	15 ml	70 ml	Deparaffinization Buffer
Buffer ATL	6 ml	30 ml	Tris/EDTA/SDS
Buffer AL	6 ml	30 ml	Guanidine Salt
Buffer GW1*	13 ml	44 ml	Guanidine Salt
Buffer GW2*	6 ml	20 ml	Tris/EDTA
Proteinase K	12 mg	50 mg	Protease
Protease Dissolve Buffer	1.8 ml	6 ml	Glycerol/Tris/CaCl2
Buffer AE	5 ml	20 ml	Tris/EDTA

Storage and stability

Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 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.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Materials and Equipment to be Supplied by User

- Heat block or water bath capable of 55℃ and 90℃
- Add 17ml (20 Preps) or 56ml (100 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 24 ml (20 Preps) or 80ml (100 Preps)80ml absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 0.6ml (20 Preps) or 2.5ml (100 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Absolute ethanol

Protocol

1. Using a scalpel, trim excess paraffin off the sample block. Cut up to 8 sections 5~10μm 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 ATL and 20µl Proteinase K into samples and vortex.** Incubate at 56℃ for 1 hours or until samples melt completely. Incubation can process overnight.

6. **Incubate at 90℃ for ~60 minutes.**

   The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.

7. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

   If RNA-free genomic DNA is required, add 10 µl RNase A (25mg/ml) and incubate for

   10min at room temperature. Allow the sample to cool to room temperature before adding RNase A.

8. **Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200µl ethanol (96–100%), and mix again thoroughly by vortexing.**

   It is essential that the sample, Buffer AL and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

9. **Insert a HiPure DNA Mini Columns I in a 2ml Collection Tube**.

10. **Transfer mixture to the column and centrifuge at 10,000 x g for 30~60 seconds.** Discard the filtrate and reuse collection tube.

    If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the column is empty.

11. **Add 650µl Buffer GW1 to the column.** Centrifuge at 10,000 x g for 30~60 senconds. Discard the filtrate and reuse collection tube.

12. **Add 650µl Buffer GW2 to the column.** Centrifuge at 10,000 x g for 30~60 senconds. Discard the filtrate and reuse collection tube.

13. Centrifuge the empty Column at 10,000 x g for 3 minutes to dry the column matrix.

14. **Transfer the Column to a clean 1.5ml centrifuge tube. Add 15~50µl Buffer AE directly to the center of the column membrane.** Stay at room temperature for 1 minutes.

Ensure that Buffer AE is equilibrated to room temperature. If using small elution volumes (<50 µl), dispense Buffer AE onto the center of the membrane to ensure complete elution of bound DNA. HiPure columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. The volume of eluate will be up to 5µl less than the volume of elution solution applied to the column. Incubating the column loaded with Buffer AE for 5 min at room temperature before centrifugation generally increases DNA yield.

15. Centrifuge at 10,000 x g for 1 minute at room temperature. Store DNA at -20℃.

Troubleshooting Guide

1. Clogged HiPure DNA Mini Column I

- **Too much samples:** reduce sample amount, paraffin sections do not over 8~10 pieces.
- **Poor lysis for sample:** Tissue sample must be cut or minced into small pieces. Increase incubation time at 56℃ to 3~6 hours to ensure tissue lysed completely.
- **Insoluble impurities in lysis buffer:** if there is still impurity particles in lysis buffer, centrifuge at 10,000 x g for 3 minutes to remove impurities completely.

2. Low or no recovery

- Refer to clogged HiPure DNA Mini Column I.
- **Incomplete crosslinking removal:** increase incubation tome at 90℃ to 90~120 minutes, remove protein crosslinking with DNA completey.
- Buffer GW1/GW2 did not add with 100% ethanol before use.
- **Poor elution:** elution buffer must be added to the middle of membrane, increase elution volume or times.
- **Poor Proteinase K activity:** prepare new Proteinase K buffer, store at -20℃.