【Product Name】HiPure Blood DNA Mini Kit

【Product specifications】20 Preps, 50 Preps, 250 Preps

【Intended Use】

This product provide fast and easy methods for purification of total DNA for reliable PCR and Southern blotting. Total DNA (e.g., genomic, viral, mitochondrial) can be purified from whole blood, plasma, serum, buffy coat, bone marrow, other body fluids, lymphocytes, cultured cells.

【Principle】

This product is based on silica column purification. The sample is lysed and digested with lysate and protease, DNA is released into the lysate. Transfer to an adsorption column. Nucleic acid is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer (10mm Tris,pH9.0, 0.5mm EDTA).

【Kit Contents】

Cat.No.	D311101	D311102	D311103
Purification Times	20 Preps	50 Preps	250 Preps
HiPure DNA Mini Columns I	20	50	2 x 125
2ml Collection Tubes	40	100	5 x 100
Buffer AL	6 ml	15 ml	60 ml
Buffer DW1	12 ml	30 ml	150 ml
Buffer GW2*	6 ml	12 ml	50 ml
Proteinase K	12 mg	24 mg	120 mg
Protease Dissolve Buffer	1.8 ml	1.8 ml	10 ml
Buffer AE	5 ml	15 ml	60 ml

【Storage conditions and Validity】

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.

【Preparation before Use】

- Add 24ml (20 Preps) or 48ml (50Preps) or 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 0.6ml (20 Preps) or 1.2ml (50Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Ethanol (96 – 100%)*
- Phosphate-buffered saline (PBS) may be required for some samples

【Protocol for Blood, Plasma and Body Fluids】

1. **Pipet 20 µl Proteinase K into the bottom of a 1.5 ml microcentrifuge tube.**
2. **Add 200 µl sample to the microcentrifuge tube. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^6 lymphocytes or Culture Cells in 200 µl PBS.**
   If the sample volume is less than 200µl, add the appropriate volume of PBS. HiPure columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 10µl of an RNase A (25 mg/ml) should be added to the sample before addition of Buffer AL.
3. **Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.**
   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. If the sample volume is larger than 200µl, increase the amount of Proteinase K and Buffer AL proportionally. Do not add Proteinase K directly to Buffer AL.
4. Incubate at 70°C for 10 min
5. Briefly centrifuge the 1.5ml microcentrifuge tube to remove drops from the inside of the lid.
6. **Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s.** After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
   If the sample volume is greater than 200µl, increase the amount of ethanol proportionally; for example, a 400µl sample will require 400µl of ethanol.
7. Insert a HiPure DNA Mini Column I into a 2mL Collection Tube(provided).
8. **Carefully apply the mixture from step 6 to the column without wetting the rim. Close the cap, and centrifuge at 10,000 x *g* for 1 min.** Place the column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

Close each spin column to avoid aerosol formation during centrifugation. Centrifugation is performed at 10,000 x *g* to reduce noise. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the mini column is empty. When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

9. Add 500µl Buffer DW1 without wetting the rim. Close the cap and centrifuge at 10000 x g for 1 min.

10. Discard the flow through and reuse the collection Tubes. Add 650µl Buffer GW2 without wetting the rim. Close the cap and centrifuge at 10000 x g for 1 min.

11. Discard the flow through and reuse the collection Tubes. Centrifuge at 10,000 x g for 1 min. This step helps to eliminate the chance of possible Buffer GW2 carryover.

12. Place the column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Add 50~200µl Buffer AE or distilled water. Incubate at room temperature for 2 min, and then centrifuge at 10000 x *g* for 1 min.
Incubating the column loaded with Buffer AE for 5 min at room temperature before centrifugation generally increases DNA yield. A second elution step with a further 50~200µl Buffer AE will increase yields by up to 15%. Elution with volumes of less than 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield). For samples containing less than 2µg of DNA, elution in 50µl Buffer AE or water is recommended. For long-term storage of DNA, eluting in Buffer AE and storing at –30 to –15°C is recommended, since DNA stored in water is subject to acid hydrolysis.

【Protocol: DNA Purification from Buccal Swabs】

1. Place buccal swab in a 2ml microcentrifuge tube and add 400µl 1% SDS to the sample. Add 20µl Proteinase K to the sample. Mix immediately by vortexing for 15 s. Incubate at 56°C for 10 min. Briefly centrifuge to remove drops from inside the lid.
2. Add 400µl Buffer AL and 400µl ethanol (96–100%) to the sample, and mix again by vortexing.
3. Follow blood protocol step 7-12 and elute DNA with 50~75µl Buffer AE.

【Preparation of buffy coat】

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500 x *g* for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

Troubleshooting Guide

1. Low or no recovery

- **Buffer GW2 did not contain ethanol:** Ethanol must be added to Buffer GW2 before used. Repeat procedure with correctly prepare Buffer.
- **Low concentration of target DNA in the Sample:** Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided. Low concentration of cells or viruses in the sample
- **Inefficient cell lysis due to insufficient mixing with Buffer AL:** Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing.
- **Low-percentage ethanol used instead of 100%:** Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

2. A260/A280 ratio for purified nucleic acids is low

- **Inefficient cell lysis due to insufficient mixing with Buffer AL:** Repeat the procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse vortexing.
- **Inefficient cell lysis due to decreased protease activity:** Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Besure to store the stock solution at -20–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer AL.
- **No ethanol added to the lysate before loading onto the column:** Repeat the purification procedure with a new sample.

3. A260/A280 ratio for purified nucleic acids is high

- **High level of residual RNA:** In future DNA preparations, use the optional RNase step included in the protocols.

4. DNA does not perform well (e.g. in ligation reaction)

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 3 min at room temperature after adding 500ul of Buffer GW2, then centriufge or Vacuum.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >10,000 x g for 1min, then dry.
- **Inappropriate elution volume used:** Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.