【Product Name】HiPure Universal DNA Kit

【Product specifications】50 Preps/Kit, 250 Preps/Kits

【Intended Use】

This product is suitable for rapid extraction of total DNA from tissue, cells, blood, saliva, swabs, blood spots, semen and other clinical samples. DNA can be used directly for PCR, quantitative PCR, Southern Blot, test of virus DNA and so on.

【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 plate and filter 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.

【Kit Contents】

Cat.No.	D301802	D301803	Main Composition
HiPure DNA Mini Columns I	50	250	Silica Column
2ml Collection Tubes	100	500	PP Column
Buffer ATL	30 ml	150 ml	Tris/EDTA/SDS
Buffer AL	30 ml	150 ml	Guanidine Salt
Buffer GW1*	22 ml	88 ml	Guanidine Salt
Buffer GW2*	12 ml	50 ml	Tris/EDTA
Proteinase K	24 mg	120 mg	Protease
Protease Dissolve Buffer	1.8 ml	10 ml	Glycerol/Tris/CaCl2
Buffer AE	15 ml	60 ml	Tris/EDTA

【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.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.

【Preparation before Use】

- Add 28ml (50 Preps) or 112ml (250 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 48ml (50 Preps) or 200 ml (250 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 1.2ml (50 Preps) or 6.0 ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Absolute ethanol

【Protocol】

A. solid tissue

1. Cut <20 mg tissue into small pieces and transfer into a new 1.5ml centrifuge tube. Add 250μl Buffer ATL and 20μl Proteinase K, shake at 55°C for 30~120 minutes or overnight until completely digested.
2. Add 250μl Buffer AL to the samples and vortex for 15 seconds. Incubation at 70°C for 10 minutes.
3. Add 250μl absolute ethanol to the samples, vortex at maximum speed for 10 seconds.

B. Anticoagulated blood (200μl)

1. Transfer 20μl Proteinase K and 200μl anticoagulated whole blood, plasma or other body fluids to a new 2ml tube, shake to mix for 5 seconds.
2. Add 200μl Buffer AL to the samples. Inverting the tube for 3~5 times, then vortex at maximum speed for 10 seconds. Incubate at 70°C for 10 minutes.
3. Add 200μl absolute ethanol to the samples, vortex at high speed for 10 seconds..

C. Saliva /plasma and other liquid samples (low DNA content)

1. Transfer 20μl Proteinase K and 500μl saliva or plasma to the tube and shake to mix for 5 seconds
2. Add 500μl Buffer AL to the samples, vortex at high speed for 10 seconds. Incubate at 65°C for 30 minutes.
3. Add 500μl absolute ethanol to the samples and vortex at high speed for 10 seconds.

D. Culture cells

1. Collect Cells (< 2 x 10^6) by centrifugation at 500 x g for 5 min. **Add 250µl Buffer PBS and 20µl Proteinase K to the sample and resuspend the cells by vortexing.**
2. **Add 250µl Buffer AL to the samples and vortex at maximum speed for 10 seconds.** Incubation at 70°C for 10 minutes.
3. Add 250µl absolute ethanol and vortex at maximum speed for 10 seconds.

E. Swab DNA extraction

1. Transfer dry swab to the 2ml centrifuge tube.
2. Add 500µl Buffer ATL and 20µl Proteinase K to the sample. Shaking at 55°C for 15 minutes.
3. Add 500µl Buffer AL and 500 µl absolute ethanol to the sample, mix well.

G. Blood stains/Seminal Spots

1. Transfer the 3 slices(3mm) to the 2.0ml centrifuge tube. **Add 500µl Buffer ATL and 20µl Proteinase K to the sample.** Shaking at high speed for 60min at 55°C.
2. **Add 500µl Buffer AL to the samples, Shaking at high speed for 10 min at 70°C.**
3. Centrifuge at 13,000 x g for 1 min. Transfer the supernatant to a new centrifuge tube, add 0.5 volume of absolute ethanol, then vortex to mix.

H. FFPE samples

1. Using a scalpel, trim excess paraffin off the sample block. Cut up to 8 sections 5–10 µm thick into a 1.5 or 2 ml microcentrifuge tube. Remove the praraffin by xylene or Buffer DPS (Deparaffinization Solution, no provided).
2. **Add 200µl Buffer ATL and 20µl Proteinase K to the sample. Incubate at 56°C for 60min, then incubate at 90°C for 60 min.**
3. Add 200µl Buffer AL and 200µl absolute ethanol to the samples, vortex to mix for 10 seconds.

Column purification

4. Insert a HiPure DNA Mini Column I in a 2ml Collection Tube.
5. **Add up to 750µl solution from Step 3 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
6. Repeat Step 5 until all of the sample has been transferred to the column.Discard the filtrate and the collection tube.
7. **Insert the column in a new 2ml Collection Tube. Add 650µl Buffer GW1to the column.** Centrifuge at 12,000 × g for 1 minute.Discard the filtrate and reuse collection tube.
8. **Add 650µl Buffer GW2 to the column.** Centrifuge at 12,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.
9. Centrifuge the empty column at 12,000 × g for 2 minute at room temperature to dry the column matrix.
10. **Place the column to a clean 1.5ml microcentrifuge tube. Add 50-200µl Buffer AE directly to the center of the column membrane.** Let sit at room temperature for 3-5 minutes.
11. Centrifuge at 12,000 × g for 1 minute at room temperature. Store DNA at -20℃.

    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.

Troubleshooting Guide

1. Low or no recovery

- **Buffer GW1/GW2 did not contain ethanol:** Ethanol must be added to Buffer GW1/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 shoµld be avoided.

2. 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 5 min at room temperature after adding 650µl 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 >12,000 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.