【Product Name】HiPure Yeast DNA Kit

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

【Intended Use】

This product provides a reliable solution for DNA isolation from yeast samples. Total DNA can be purified from yeast (<5x 10^7) without phenol or chloroform. The whole extraction can be finished within 60 minutes. Purified DNA can be directly used for PCR, Southern blot, ect.

【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.	D314701	D314702	D314703
Purification Times	20	50	250
HiPure DNA Mini Columns I	20	50	250
2ml Collection Tubes	20	50	250
Glass Beads (0.4~0.6mm)	10 g	20 g	90 g
Buffer SE	12 ml	30 ml	150 ml
Lyticase	1.0 ml	1.8 ml	5 x 1.8 ml
Proteinase K	6 mg	12 mg	60 mg
Protease Dissolve Buffer	1.8 ml	1.8 ml	5 ml
Buffer ATL	15 ml	30 ml	150 ml
Reagent DX	200 ul	500 ul	1500 ul
Buffer DL	15 ml	30 ml	150 ml
Buffer GW1*	6.6 ml	13 ml	66 ml
Buffer GW2*	10 ml	20 ml	2 x 50 ml
Buffer AE	5 ml	15 ml	30 ml
Protocol	1	1	1

【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 dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. Buffer ATL may precipitate at low temperature. Dissolve it by 37℃ water bath.

【Preparation before Use】

- Add 8.4ml (20Preps) or 17ml (50Preps) or 84ml (250 Preps) absolute ethanol to the bottle of Buffer GW1.
- Add 40ml (20Preps) or 80ml (50Preps) or 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2.
- Add 0.3ml (20Preps) or 0.6ml (50Preps) or 3.0ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Ethanol (96 – 100%)

【Standant Protocol】

1. **Transfer 1-1.5ml yeast cultures to 2.0ml centrifuge tube, centrifuge at 5,000 x g for 5 min to collect yeasts and remove the supernatant.**
2. **Add 300µl buffer SE, 10µl 2-Mercaptoethanol and 30µl Lyticase to the precipitate.** The precipitate was fully suspended by vortex and oscillating incubation at 37℃ for 30~60 minutes.
3. **Centrifuge at 5,000 x g for 5 min.** Remove the supernatant.
4. **Add 300µl buffer ATL to the precipitate.** The precipitate was fully suspended by vortex.
   If RNA-free genomic DNA is required, 10µl of RNase A (no provided) should be added to the sample and incubate for 15 minutes at room temperature.
5. **(Optional) Add ~300mg glass beads and 2µl Reagent DX to the sample and vortex at maximum speed for 5min for further lysis of yeast cells**
6. **Add 300µl Buffer DL and 10µl Proteinase K to the sample.** Fully vortex and incubate at 70°C by water bath for10 min.
7. **Centrifuge at 10,000 x g for 3 min to remove undigested impurities.** Transfer 500µl supernatant to a new centrifuge tube.
8. **Add 250µl absolute ethanol to the supernatant and vortex for 10s.**

9. Insert a HiPure DNA Mini Column I into a 2mL Collection Tube (provided). **Transfer the mixture to the column. Close the cap and centrifuge at 10,000 x g for 30~60s.**

10. **Discard the flow through and reuse the collection Tubes. Add 500µl Buffer GW1 (diluted by absolute ethanol). Close the cap and centrifuge at 10,000 x g for 30~60s.**

11. **Discard the flow through and reuse the collection Tubes. Add 600µl Buffer GW2 (diluted by absolute ethanol). Close the cap and centrifuge at 10,000 x g for 30~60s.**

12. Discard the flow through and reuse the collection Tubes. Centrifuge at 13,000 x g for 2 min. This step helps to eliminate the chance of possible Buffer GW2 carryover.

13. **Place the column in a clean 1.5 ml centrifuge tube. Add 30~50µl Buffer AE (Pre-heated to 65℃) to the middle of the membrane). Incubate at room temperature for 3 min, and then centrifuge at 10000 x g for 1 min.**
    Incubating the column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield. A second elution step with a further 100~200µl Buffer AE will increase yields by up to 15%.

14. **Discard the column and store DNA at 2-8℃. For long term it should be stored at -20℃ or -80℃.**

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.
- **Inefficient cell lysis due to insufficient mixing with Buffer DL:** Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer DL immediately and thoroughly by pulse-vortexing.

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

- **Inefficient cell lysis due to insufficient mixing with Buffer DL:** 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. Be sure to store the stock solution at -20–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer DL.
- **No ethanol added to the lysate before loading onto the column:** Repeat the purification procedure with a new sample.

3. 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 centrifuge 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.