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Yeast DNA Extraction Kit Instructions

Composition

Yeast DNA Extraction Kit Cat. No.	5 Preps 3401005	50 Preps 3401050	250 Preps 3401250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
RNaseA	15 µl	120 µl	600 µl
Protease K	120 µl	1.2 ml	1.2 ml×5
Buffer AT	5 ml	40 ml	200 ml
Buffer K	2 ml	20 ml	100 ml
Buffer WA (concentrate)	1.9 ml	12 ml	60 ml
Buffer WB (concentrate)	1.5 ml	10 ml	50 ml
Buffer TE	1.2 ml	6 ml	30 ml
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Storage

1. Protease K and RNaseA can be transported at room temperature. Please store protease K at -20°C and RNaseA at 2-8°C.
2. All the reagents and components can be stored for up to two years at room temperature (0~30°C). For longer storage, it is recommended to keep at 2-8°C.
3. The product stored at 2~8°C should be restored to room temperature before use.

Technical Support

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Introduction

This product is suitable for extracting total DNA from 5~10 ml yeast cell culture solution. The yeast cells were frozen in liquid nitrogen, ground and broken, and the genome DNA was released by adding Buffer AT and protease K, the impurities such as protein and polysaccharide of the yeast cells were precipitated by Buffer K. After the supernatant containing DNA is added to the Spin Column, the DNA is bound to the Spin Column, and the remaining proteins and PCR inhibitors are filtered out. The DNA is washed by Buffer WA and Buffer WB and eluted with Buffer TE, which can be used for various molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. Liquid nitrogen, mortar, or sample cracking tube M (Simgen Cat. No C-001-2).
3. 1.5 ml centrifuge tube, pipette, and tips.
4. Latex gloves, disposable masks and other protective items and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.
7. Water bath.

Preparation Before Use

1. If the centrifuge has refrigeration function, please set the temperature to 25°C.
2. Set the water bath temperature to 70°C and incubate Buffer AT and Buffer TE to 70°C.
3. Add Absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".

Protocol

1. **Collect 5~10 ml of yeast liquid cultured overnight and discard the supernatant.**

* If yeast is collected in a 1.5 ml centrifuge tube, the yeast can be enriched by centrifugation several times at 12000 rpm for 30 sec.

2. **Add 100 µl Buffer AT incubated at 70℃ to the pellet, pipette several times directly with the tip and transfer it into the mortar. Add the liquid nitrogen to the yeast suspension (yeast suspension will immediately agglutinate after touching liquid nitrogen) and grind it into powder form.**

* If the yeast does not grind into a powder, add liquid nitrogen to continue grinding, otherwise it will seriously affect the recovery efficiency of the final DNA.
* In the absence of liquid nitrogen, the cell wall can be fully broken by direct grinding for about 15 min (if the liquid evaporates during this process, add 100 µl Buffer AT to continue grinding) or by using the grinding sample cracking tube M (Simgen Cat. No C-001-2).

3. **When the powdered yeast begins to thaw, add 500 µl Buffer AT and 2 µl RNase A to the mortar, continue grinding several times, and transfer the lysate into a 1.5 ml centrifuge tube and incubate at 70℃ for 5 min.**

* If the lysate is less than 500 µl, add Buffer AT to 500 µl.

4. **Add 20 µl protease K, vortex for 30 sec, and incubate at 70℃ for 20 min. Invert the tube several times every 2~3 min to facilitate the release of DNA.**
5. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec and mix well. Centrifuge at 13,000 rpm for 5 min.**
6. **Transfer the supernatant from step 5 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid and centrifuge at 12,000 rpm for 30 sec.**
7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that Absolute ethanol has been added to Buffer WA.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that Absolute ethanol has been added to Buffer WB.

9. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, as this will cause problems in downstream applications due to the residual ethanol in the eluate.

10. **Discard 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 80-100 µl Buffer TE incubated at 70℃ into the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof cover, change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the tube cover falling off.

11. **Discard the Spin Column. The eluted DNA can be used for various molecular biology experiments or stored at -20℃ for later use.**