

Yeast DNA Fast Extraction Kit (Spin-Column)



DP 6201 (50 preps)

Kit Content, Storage Condition and Stability

Content	Storage	50 preps
Enzyme B	-20°C	1 g
Proteinase K	-20°C	1,5 ml
Buffer CB	RT	15 ml
Inhibitor Removing Buffer IR	RT	25 ml
Washing Buffer WB	RT	15 ml
Elution Buffer EB	RT	5 ml
Spin Column AC	RT	50
Collection Tubes 2 ml	RT	50

All reagents, when stored properly, are stable for 12 months.

Note:

1. Sorbitol buffer: 0.1M Na₂HPO₄ (pH7.4)+ 1.2 M Sorbitol, storage at 4°C
2. 0.1M Na₂HPO₄(pH 7.4): 77.4ml 0.1mol/L Na₂HPO₄ + 22.6ml 0.1mol/L Na₂HPO₄.
3. **Dilute Washing Buffer WB with absolute ethanol before use and mark it on the label.**

Principle

In this kit, innovative extraction and lysis system allows rapid lysis of cells and inactivating cellular nucleases. The treatment of glass beads is eliminated and the integrity of genomic DNA purity is greatly ensured.

Features

1. Rapid, DNA isolation under 30 min, isolated DNA is longer than 50 Kb
2. Compatible, suitable for various yeast and fungi
3. High purity, purified DNA has typically A₂₆₀/A₂₈₀ ratio between 1.7 and 1.9;
4. DNA can be directly used for most downstream applications, including PCR, Southern blot, restriction digestion etc.
5. High yield.15 - 30 ug DNA isolated from 3 ml of the yeast culture in log phase

Procedure

1. Harvest yeast culture (less than 5×10⁷ cells). Centrifuge at 12.000 rpm for 1 min. Resuspend the pellet with 600 µl of Sorbitol buffer.
2. Add 20 mg Enzyme B, mix thoroughly and then incubate at 37°C in water bath for 30-60 min.
3. Centrifuge at 4.000 rpm for 5 min, discard supernatant and resuspend the pellet with 200 ul of PBS buffer.
4. Add 20-30 ul Proteinase K (20 mg/ml) and 220 ul buffer CB, mix thoroughly. Incubate at 70°C in water bath for 10 min until solution become clear.
5. Add 220 ul absolute ethanol, mix thoroughly. Centrifuge at 12.000 rpm for 1 min.
6. Transfer supernatant to the Spin-column AC and wait for 1 min. Centrifuge at 12.000 rpm for 1 min. Discard the waste liquid.
7. Add 500 µl Buffer IR and centrifuge at 12,000rpm for 30 sec. Discard the waste liquid.
8. Add 700 µl Buffer WB (**please dilute with absolute ethanol before use**) and centrifuge at 12.000 for 30 sec. Discard the waste liquid.
9. Add 500 µl Buffer WB and centrifuge at 12,000 rpm for 30 sec. Discard the waste liquid.
10. Put the Spin-colum AC back to the collection tube and centrifuge at 13.000 rpm for 2 min. Remove rinsing buffer as possible, as the left ethanol can affect the next reaction .
11. Transfer the Spin-column AC to a new collection tube and add add 50 ul of preheated (65°C-70°C) Buffer EB. Let it stay at room temperature for 2-5 min. Centrifuge at 12.000 rpm for 1 min. Add the flow-through back into Spin Column AC and let it stay at room temperature for 2 min. Centrifuge at 12.000 rpm for 1 min.
12. Store eluted DNA at -20°C.