# Rapid Yeast Genomic DNA Extraction Kit

**Code No.:** NG421S 100T

NG421M 200T

| Contents | storage | 100 T | 200T |
|---|---|---|---|
| lysozyme | -20 ℃ | 25 mg | 50 mg |
| Proteinase K | -20 ℃ | 2×20 mg/ml | 4×20 mg /ml |
| Buffer YTL | RT | 20 ml | 40 ml |
| Buffer CB | RT | 20 ml | 40 ml |
| Buffer IR | RT | 50 ml | 100 ml |
| Buffer WB | RT | 25 ml | 50 ml |
| | | Add absolute alcohol before use | |
| Buffer EB | RT | 15 ml | 20 ml |
| Spin Columns AC/ Collection tubes | RT | 100 | 200 |

**Important Note:**

1. All solutions should be clear. If the ambient temperature is low, the solutions may form precipitates and should not be used directly. In such cases, they be heated in a 37 °C water bath for a few minutes to restore clarity. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure the reagents to air, the caps of each solution should be tightly closed immediately after use.
2. Sorbitol buffer: 1.2 M sorbitol prepared with 0.1 M sodium phosphate buffer (pH 7.4): 77.4 ml of 0.1 M sodium phosphate buffer (pH 7.4) and 22.6 ml of 0.1 mol/L NaH2PO4; take 30 ml of sorbitol buffer to dissolve lysozyme in the 100-test kit, and so on, or add as needed for each experiment.

**Introduction:**

This kit utilizes a DNA adsorption column and a novel, unique solution system, making it suitable for the rapid and simple extraction of genomic DNA from fungal tissue. The purification of one or more 100 mg fresh or 20 mg dried Yeast samples can be completed within 30 minutes. The extraction process does not require organic solvents such as phenol-chloroform, nor does it require time-consuming isopropanol or ethanol precipitation. It can rapidly and efficiently remove impurities such as polysaccharides, phenols, enzyme inhibitors. The purified DNA can be directly used for experiments such as PCR, restriction enzyme digestion, and hybridization.

A unique lysis buffer rapidly lyses cells, DNA is adjusted for binding conditions, it is selectively adsorbed onto silica matrix membrane in the spin column under high-chaotropic salt conditions. Then, through a series of rapid wash-and-centrifugation steps, impurities such as cellular and proteins are removed. Finally, low-salt water elutes the purified DNA from the silica matrix membrane.

**Safety Information:**

1. Lysis buffer YTL, CB and IR contain irritating and harmful compounds. Wear latex gloves during operation to avoid contact with skin, eyes and clothing. If contact with skin or eyes occurs, rinse with plenty of water or saline.
2. Simple and fast, single sample operation can generally be completed within 1 h, the simplest and fastest kit in the world.
3. Multiple rinsing steps to remove proteins result in higher DNA purity.
4. Pre-warm the required water bath to 70 °C before starting the experiment.

5. All centrifugation steps are performed at room temperature, and the centrifuge speed must reach 13,000 rpm.

**Operation Procedure:**

**Tip:** Before the first use, please add absolute alcohol in the buffer WB and mark it.

1. Harvest Yeast cells (at most no more than $5\times10^7$ cells) in a microcentrifuge tube by centrifugation at 12,000 rpm for 1 min at room temperature (15-30 °C), then remove all traces of supernatant until all medium has been drained.
2. Add 600 µl Sorbitol buffer and 0.25 mg of lysozyme, mix thoroughly. Placed at 30 °C for 30 min, centrifugation at 4,000 rpm for 10 min, then remove all traces of supernatant.
3. Add 180 µl buffer YTL and 20 µl Proteinase K (20 mg/ml), mix and vortex thoroughly. Placed at 56 °C for 1 h.
4. Add 200 µl buffer CB and 20 µl RNase A (10 mg/ml), mix and vortex thoroughly. Placed at 70 °C for 10 min.
5. After cooling, add 100 µl isopropanol, vortex immediately to mix thoroughly, at which time flocculent may appear.
6. Use a 1 ml pipette tip to aspirate the mixture, add the mixture to an adsorption column AC (place the adsorption column in a collection), centrifuge at 13,000 rpm for 60 seconds, and discard the waste liquid in the collection tube.
7. Add 500 µl buffer IR, Centrifuge for 30-60 sec at 12,000 rpm. Discard the flow-through and set the Spin Column AC back into the Collection Tube.
8. Add 600 µl of Buffer WB to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
9. Repeat step 8.
10. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
11. Add 30-80 µl of Buffer EB or water to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min at 12,000 rpm (The elution buffer can be preheated at 65-70 °C to increase the elution effect). Note: The volume of elution buffer should not be less than 30 µl, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.
12. DNA can be stored at 2-8 °C, and for long-term storage, it can be kept at -20 °C