Quick Yeast Genomic DNA Extraction Kit

Item N°: NB-03-0029 50 preps
NB-03-0030 100 preps

Kit Content

Content	NB-03-0029	NB-03-0030
Solution DS	15 ml	30 ml
Solution MS	20 ml	40 ml
Proteinase K	1 ml	2 ml
Wash Buffer PS	30 ml	60 ml
Wash Buffer PE	15 ml	30 ml
Eluent Buffer TE	5 ml	10 ml
Spin Column	50 each	100 each

Description

The Yeast Genomic DNA Extraction Kit provides a simple and rapid method for high quality genomic DNA purification from yeast. The Yeast Genomic DNA system uses the silica-gel-membrane technology for simple and fast isolation of Genomic DNA without phenol/chloroform. Homogenization is not necessary since tissues are directly lysed by Proteinase K. The buffer system is optimized to allow selective binding of DNA to the silica-gel membrane. The simple centrifugation protocol completely removes contaminants such as proteins, divalent cations, and secondary metabolites. Pure DNA is then eluted in water or low-salt buffer, ready to use. The typical yield of genomic DNA is 3-25 µg from 1–5 x 10^7 yeast cells. The purified high molecular weight genomic DNA is suitable for direct use in all common molecular biology applications: PCR, restriction digestion, cloning, DNA sequencing and Southern blot analysis.

Features

Efficient: 3-35 µg of genomic DNA from 1 – 5 x 10^7 yeast cells.

Fast: Procedure takes only 1 h.

Safe: No phenol/chloroform extraction step.

High purity: Purified DNA is ready for downstream application such as PCR, restriction digestion.

Downstream Applications

Purified DNA is free from contaminants and enzyme inhibitors, and typically has A_{260}/A_{280} ratios between 1.7 and 1.9, and is suitable for applications such as:

- Restriction digestion
- PCR
- Labeling
- Library construction

Storage

Store Protein K at -20°C, other reagents can be stored at room temperature for up to 1 year. Any precipitate in the Solution DS and Solution MS can be re-dissolved by incubating at 37°C before use.

Important Notes

- Prior to the initial use of the kit, dilute the Wash Buffer(PE) with ethanol (96-100%):

	NB-03-0029 (50preps)	NB-03-0030 (100preps)
Wash Buffer(PE)	15 ml	30 ml
Ethanol	45 ml	90 ml
Total Volume	60 ml	120 ml

After the ethanol has been added, mark the check box on the bottle to indicate the completed step.

- Examine the solution for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.
- All purification steps should be carried out at room temperature.

Protocol

1. Add 1 -5 ml of an yeast culture (not exceed 5 x 10^7 cells) to a 1.5ml microcentrifuge tube. Centrifuge at 12,000 rpm for 12 min to pellet the cells. Remove the supernatant.

2. Add 600 µl lyticase buffer and 200 U lyticase, mix thoroughly by vortexing, incubate at 30 °C for 30 minutes.

Note : Lyticase need to be supplied by the user.

3. Centrifuge at 5,000 rpm for 8–10 min to pellet the mixture. Romove the supernatant. Add 200 µl Solution DS to the pellet. It is essential that the sample and Solution DS are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

Optional : If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 5 min at room temperature. RNase A (100 mg/ml) can be purchased separately .

4. Add 20 µl Proteinase K and 220 µl Solution MS. Mix thoroughly by vortexing. Incubate at 65°C for 10 minutes to yield a homogeneous solution. Spin down the water beads on the wall of the tube.
5. Add 220 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol are mixed thoroughly. A precipitate may appear. Pipet the mixture from step 4 into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 12,000rpm for 1 min. Discard flow-through.
6. Add 500 µl Wash Buffer PS, and centrifuge for 1 min at 12,000 rpm.Discard flow-through.
7. Add 500 µl Wash Buffer PE, and centrifuge for 1 min at 12,000 rpm. Discard flow-through. Repeat step 6 again.
8. Centrifuge for 3 min at 12,000 rpm to dry the column membrane. Discard flow-through and collection tube.

Note : It is important to dry the membrane of the spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.Following the centrifugation step, remove the spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 12,000 rpm.

9. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Eluent Buffer AE (prewarm to 65°C)directly onto the membrane. Incubate at room temperature for 2 minutes, and then centrifuge for 2 min at 12,000 rpm to elute. The tube contains the purified DNA. Store the DNA at -20°C.