NZY Tissue gDNA Isolation kit

Catalogue number: MB13502, 50 columns
MB13503, 50 columns

Support protocol for isolating genomic DNA from yeast

I. Sample preparation

Harvest 3 ml of yeast culture (OD_{600} up to 10) by centrifugation for 10 min at 5,000 x *g*. Wash the cells once with 1 mL 10 mM EDTA, pH 8. Remove the supernatant and pellet the cells by centrifugation at 5,000 x g for 10 min.

II. Pre-lysis of sample

Ressuspend the pellet in 600 µL sorbitol buffer (1.2 M sorbitol; 10 mM $CaCl_2$; 0.1 M Tris/Cl pH 7.5; 35 mM β-mercaptoethanol).

Add 50 U lyticase or zymolase and incubate at 30 °C for 30 min (***note****: this step degrades the yeast cell wall creating spheroplasts. Spheroplast formation may be checked microscopically*).

Centrifuge the mixture for 10 min at 2,000 x g, remove supernatant and resuspend the pelleted spheroplasts in 180 µL buffer NT1.

Add 25 µL Proteinase K solution to the sample. Mix thoroughly by vortex. Incubate at 56 °C for 1-3 hours and vortex occasionally during incubation (***note****: samples that are difficult to lyse can be incubated overnight as well).*

III. Removal of RNA (optional)

If RNA-free DNA is required, add 20 µL of RNase A solution (20 mg/µL) to each sample. Mix and incubate for 5 min at room temperature.

Proceed with step 4 of the standard protocol.