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NucleoSpin® Plasmid – purification of plasmid DNA from yeast (Rev. 01)

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure. The latest version of the user manual is available at ***www.mn-net.com/usermanuals*** *or can be requested from our technical service (tech-bio@mn-net.com). Material safety data sheets (MSDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

Additional reagents needed:

- Wash Buffer: 10 mM EDTA, pH 8.0
- Sorbitol Buffer (prepare fresh): 1.2 M sorbitol, 10 mM $CaCl_2$, 0.1 M Tris/HCl pH 7.5, 35 mM ß-mercaptoethanol
- Lyticase or zymolase

1 Harvest yeast cells

Centrifuge **3 mL YPD yeast culture** (OD_{600} up to 10) for **10 min** at **5,000 x *g*** and discard supenatant.

2 Wash cells

Resuspend cells in **1 mL Wash Buffer**. Centrifuge for **10 min** at **5,000 x *g*** and discard supernatant.

3 Resuspend pellet

Resuspend cells in **600 µL Sorbitol Buffer**.

4 Digest cell wall

Add **200 U lyticase or zymolase** and incubate for **30 min** at **30 °C** (spheroplast formation).

5 Isolate spheroplasts

Centrifuge for **10 min** at **2,000 x *g*** and discard supernatant.

6 Isolate plasmids

Continue with step 2 of the NucleoSpin® Plasmid standard protocol with addition of **250 µL Buffer A2**.

Important: During potassium / SDS precipitation, cell wall components as well as associated genomic DNA and proteins are precipitated. Lysis should be performed with utmost care (specially step 2 of the standard protocol), as in contrast to bacterial cells, genomic DNA is not associated to the cell wall and therefore, its separation becomes more difficult.

Note: This protocol can as well be used in the 8-well or 96-well format.