NucleoSpin® Plasmid – purification of plasmid DNA from *Agrobacterium tumefaciens* (Rev. 01)

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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 reagent needed:

- Lysis Buffer: 25 mM Tris/HCl, pH 8.0, 10 mM EDTA pH 8.0, 50 mM glucose, and 50 mg/mL lysozyme
- *Optional*: *100 µg/mL RNase A*
- Wash Buffer: 1 M NaCl

1 Grow cells

Inoculate **4 mL** of **culture medium** (YEB (Yeast Extract Broth)+ antibiotics) and incubate culture for **36–48 h** at **28 °C** (medium gets highly viscous).

2 Harvest cells

Aliquot the medium in 2 mL tubes, centrifuge at **14,000 x *g*** for **1 min** at **RT**, and discard supernatant.

3 Wash cells

Resuspend cells in **1 mL Wash Buffer**, centrifuge at **14,000 x *g*** for **1 min** at **RT**, and discard supernatant.

4 Digest cell wall

Resuspend cells in **200 µL Lysis Buffer** and shake for **30 min** at **37 °C**.

5 Isolate plasmid DNA

Fill up to a total volume of 500 µL with **Resuspension Buffer A1**.

Continue with step 2 ('Cell lysis') of the NucleoSpin® Plasmid protocol for low-copy plasmids with the addition of **500 µL Lysis Buffer A2**.