NucleoBond® PC 10000 – low copy plasmid purification (Rev. 02)

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****.*

1 Cultivate and harvest bacterial cells

Harvest bacteria from **1–4 L saturated LB culture** by centrifugation at **4,500–6,000 x *g*** for **15 min** at **4 °C**.

2 Cell lysis

Carefully resuspend the pellet of bacterial cells in **240 mL Buffer S1 + RNase A**.

Add **240 mL Buffer S2** to the suspension. Mix gently by inverting the tube 6–8 times. Incubate the mixture at room temperature (18–25°C) for 2–3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

Add **240 mL pre-cooled Buffer S3 (4 °C)** to the suspension. Immediately mix the lysate gently by inverting the flask 6–8 times until a homogeneous suspension containing an off-white flocculate is formed. Incubate the suspension on ice for 5 min.

3 Equilibration of the column

Equilibrate a NucleoBond® AX 10000 (Giga) Column with **100 mL Buffer N2**. Allow the column to empty by gravity flow. Discard flow-through.

4 Clarification of the lysate

Clear the lysate by following either **option 1** or **option 2**, described below. This step is extremely important; excess precipitate left in suspension may clog the NucleoBond® column in later steps.

Option 1. Filter the suspension.

NucleoBond® PC 10000 (Giga) kits contain two types of folded filters (type 1 and type 2) in order to guarantee an optimal and fast filtration. Put Folded Filter of type 1 into Folded Filter of type 2 and place then in a large funnel for support. Prewet the filters with few drops of Buffer N2 or sterile deionized H_2O before loading lysate.

Clear the lysate as described in step 4 of the NucleoBond® PC 10000 protocol in section 7.3 'High-copy plasmid purification (Mega, Giga)' and see additional information on lysate clarification in section 3.5 when using Folded Filters.

Alternatively, the vacuum operated NucleoBond® Bottle Top Filters (not included) can be used for the filtration of the lysate.

Option 2. Centrifuge the suspension.

Centrifuge at > 12,000 x *g* for the minimum time of 60 min at 4 °C. If the suspension contains residual precipitate after the first centrifugation, either repeat this step or proceed with option 1.

Proceed with the protocol for high-copy plasmid purification at step 5 ('Binding').