5 NucleoSpin® Plasmid / Plasmid (NoLid) protocols

5.1 Isolation of high-copy plasmid DNA from *E. coli*

Before starting the preparation:

- Check if Wash Buffer A4 was prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use **1–5 mL** of a saturated ***E.coli* LB culture**, pellet cells in a standard benchtop microcentrifuge for **30 s** at **11,000 x *g***. Discard the supernatant and remove as much of the liquid as possible.

11,000 x *g*, 30 s

Note: For isolation of low-copy plasmids refer to section 5.2.

2 Cell lysis

Add **250 µL Buffer A1**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

+ 250 µL A1

Resuspend

Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30–40 °C until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature (18–25 °C).

+ 250 µL A2

Mix

RT, 5 min

Add **250 µL Buffer A2**. Mix gently by inverting the tube **6–8 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

Add **300 µL Buffer A3**. Mix thoroughly by inverting the tube **6–8 times** until blue samples turn colorless completely! Do not vortex to avoid shearing of genomic DNA!

+ 300 µL A3

Mix

Make sure to neutralize completely to precipitate all protein and chromosomal DNA. LyseControl should turn completely colorless without any traces of blue.

3 Clarification of lysate

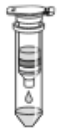
Centrifuge for **5 min** at **11,000 x *g*** at room temperature.

Repeat this step in case the supernatant is not clear!

11,000 x *g*, 5–10 min

4 Bind DNA

Place a NucleoSpin® Plasmid/Plasmid (NoLid) Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum of 700 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x *g***. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the collection tube.



Load supernatant

11,000 x *g*, 1 min

Repeat this step to load the remaining lysate.

5 Wash silica membrane

Add **600 µL Buffer A4** (supplemented with ethanol, see section 3). Centrifuge for **1 min** at **11,000 x *g***. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the **empty** collection tube.

+ 600 µL A4

11,000 x *g*, 1 min

6 Dry silica membrane

Centrifuge for **2 min** at **11,000 x *g*** and discard the collection tube.

<u>Note:</u> Residual ethanolic wash buffer might inhibit enzymatic reactions.

11,000 x *g*, 2 min

7 Elute DNA

Place the NucleoSpin® Plasmid/Plasmid (NoLid) Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE**. Incubate for **1 min** at **room temperature**. Centrifuge for 1 min at **11,000 x *g***.

+ 50 µL AE

RT, 1 min

11,000 x *g*, 1 min

<u>Note:</u> For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.5.