Supplementary protocol

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NucleoSpin® Plasmid Transfection-grade – isolation of high-copy plasmid DNA using a vacuum manifold (Rev. 01, October 2016)

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). Safety data sheets (SDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

1 Cultivate and harvest bacterial cells

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

1–5 mL *E. coli* culture

11,000 x *g*, 30 s

2 Lyse cells

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 any precipitate is dissolved. Mix thoroughly and cool buffer down to room temperature (18–25 °C).

+ 250 µL A2

Mix gently

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 a maximum of **5 min** or until the lysate appears clear.

+ 350 µL A3
Mix

Add **350 µL Buffer A3**. Mix thoroughly by inverting the tube **until the blue samples turn colorless completely**! Do not vortex to avoid shearing of genomic DNA!

3 Clarify lysate

Centrifuge for **10 min** at **> 11,000 x g** at **room temperature**.

Repeat this step in case the supernatant is not clear!

11,000 x *g*, 10 min

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4 Bind DNA

Place a **NucleoSpin® Plasmid TG Column** onto a suitable vacuum manifold with Luer-connections like the NucleoVac 24 Vacuum Manifold and **load up to 700 µL supernatant**. Do not close the lid!

Load supernatant

Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

-0.2 to -0.4 bar*, 1 min

When the sample has passed the NucleoSpin® Plasmid TG Column, release the vacuum.

If necessary, load remaining sample and repeat the step.

5 Wash silica membrane

Add **700 µL Buffer ERB**. Apply vacuum of **-0.2 to -0.4 bar* (1 min)**. When the buffer has passed the NucleoSpin® Plasmid TG Column, release the vacuum.

+ 700 µL ERB

-0.2 to -0.4 bar*, 1 min

Add **650 µL Buffer AQ** (supplemented with ethanol, see section 3). Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

+ 650 µL AQ

When the buffer has passed the NucleoSpin® Plasmid TG Column, release the vacuum.

-0.2 to -0.4 bar*, 1 min

* Reduction of atmospheric pressure

6 Dry silica membrane

-0.4 to -0.6 bar*, 5 min

Option 1: Drying by vacuum

Apply vacuum of **-0.4 to -0.6 bar*** for **5 min** to remove **Buffer AQ** completely. Run the vacuum pump continuously. Achieving and keeping a continuous air flow is more important than reaching the exact mentioned reduction of atmospheric pressure. Do not close the lid!

Release the vacuum.

Option 2: Drying by centrifugation

Place the NucleoSpin® Plasmid TG Column into a Collection Tube (2 mL). Centrifuge for **1 min** at **11,000 x *g*** to remove Buffer AQ completely. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the collection tube.

11,000 x *g*, 1 min

Note: Residual ethanol from Buffer AQ might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

7 Elute DNA

+ 50 µL AE

Place the NucleoSpin® Plasmid TG Column into a new 1.5 mL microcentrifuge tube (not provided).

Add **50 µL Buffer AE** and incubate at **room temperature** (18–25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 x *g***.

RT, 1 min

11,000 x *g*, 1 min

* Reduction of atmospheric pressure