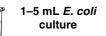


# NucleoSpin<sup>®</sup> Plasmid Transfection-grade – isolation of high-copy plasmid DNA using a vacuum manifold (Rev. 01, October 2016)

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.



11,000 x <i>g</i> ,	
30 s	

+ 250 µL A1

Resuspend

+ 250 µL A2

Mix gently

RT, 5 min

+ 350 µL A3

Mix

# 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!

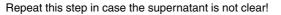
Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30-40 °C until any precipitate is dissolved. Mix thoroughly and cool buffer down to room temperature (18–25 °C).

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.

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.







### **Bind DNA** 4 Load Place a NucleoSpin® Plasmid TG Column onto a supernatant suitable vacuum manifold with Luer-connections like the NucleoVac 24 Vacuum Manifold and load up to 700 µL supernatant. Do not close the lid! -0.2 to -0.4 bar\*, Apply vacuum of -0.2 to -0.4 bar\* (1 min). 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 + 700 µL ERB 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 -0.2 to -0.4 bar\*, vacuum. 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

<sup>\*</sup> Reduction of atmospheric pressure

-0.4 to -0.6 bar\*,

5 min

11,000 x g,

1 min

Ò

#### Dry silica membrane 6

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.

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		
	Place the NucleoSpin <sup>®</sup> Plasmid TG Column into a new 1.5 mL microcentrifuge tube (not provided).		+ 50 μL ΑΕ
	Add 50 $\mu$ L Buffer AE and incubate at room temperature (18–25 °C) for 1 min. Centrifuge for 1 min at 11,000 x g.	9	RT, 1 min
		Ò	11,000 x <i>g</i> , 1 min

<sup>\*</sup> Reduction of atmospheric pressure