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NucleoSpin® 8/96 Tissue – purification of DNA from up to 5 x 10^6 cultured cells (Rev. 01)

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 equipment needed:

- PBS (dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 in 800 mL H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 liter)
- Water bath or heating block
- Appropriate lysis vessel (e.g., Round-well Block, REF 740761)

Additional preparations before starting:

- Heat a water bath or heating block to 70 °C.

1 Prepare samples

Resuspend **up to 5 x 10^6 cultured cells** in a final volume of **200 µL PBS**.

2 Lyse cells

Transfer **25 µL Proteinase K** solution and **180 µL Buffer T1** to each lysis vessel containing the resuspended cells. Mix by pipetting up and down (10 cycles).

Incubate the vessel containing the samples at **70 °C** for **1 h** until the cells are completely lysed. For optimal lysis, mix occasionally during incubation. Make sure that the lysis vessels are securely closed.

Centrifuge the vessel (15 s; 1,500 x *g*) to collect any condensate from the lid of the vessel.

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3 Adjust DNA binding conditions

Add **400 µL Buffer BQ1** and **400 µL 96–100 % ethanol** to each sample. Mix by vigorous shaking for 10–15 s. Spin briefly (10 s; 1,500 x *g*) to collect any sample from the Cap Strips.

Using increased volumes of lysis buffers minimizes the risk of clogging of the silica membrane in the NucleoSpin® Tissue Binding Strips / Plates.

Proceed with step 4 of the NucleoSpin® 8 / 96 Tissue standard protocol ('Transfer lysates').