# Using NucleoSpin® 8/96 Tissue for purification of DNA from up to 5 x $10^6$ cultured cells (Rev. 02)

Before starting with the preparation, set incubator or oven to 70°C. Equilibrate Buffer BE to 70°C. Prepare Buffer B5 and Proteinase K solution according to the instructions in the NucleoSpin® 8/96 Tissue user manual (section 3).

For resuspension of the cells from the use of an appropriate lysis vessel, e.g. Round-well Block is recommended (REF 740761). )

## Procedure

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

**2. Lyse samples.**
Transfer 25 µL of Proteinase K solution and 180 µL of 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 × *g*) to collect any condensate from the lid of the vessel.

**3. Adjust DNA binding conditions.**
Proceed with **step 2** of the **NucleoSpin® 8/96 Tissue protocol** by using **doubled volumes of Buffer BQ1 and ethanol** (400 µL each).
Using increased volumes of lysis buffers minimizes the risk of clogging of the silica membrane in the NucleoSpin® 8 Tissue Binding Strips / NucleoSpin® 96 Tissue Binding Plates.



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# Support protocol



## Optional: Purification of DNA from up to 1 x $10^6$ cultured cells using the NucleoSpin® 8/96 Tissue (Rev. 02)

Before starting with the preparation, set incubator or oven to 70°C. Equilibrate Buffer BE to 70°C. Prepare Buffer B5 and Proteinase K solution according to the instructions in the NucleoSpin® 8/96 Tissue user manual (section 3).

For resuspension of the cells from the use of an appropriate lysis vessel, e.g. Round-well Block is recommended (REF 740761). )

**Prodecure**

**1. Prepare samples.**
Resuspend **< 1 x $10^6$ cultured cells** in a final volume of 200 µL Buffer T1. The use of an appropriate lysis vessel.

**2. Prepare samples.**
Transfer 25 µL of Proteinase K solution 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 × g) to collect any condensate from the lid of the vessel.

**3. Adjust DNA binding conditions.**
Proceed with **step 2 of the NucleoSpin® 8/96 Tissue protocol** (using the volumes of Buffer BQ1 and ethanol noted in the protocol).



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