NucleoSpin® 96 DNA Plasma – Recommendations for sample lysis in 96- or 24-well plates (Rev. 01, November 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****.*

The procedures below describes the isolation of cell-free DNA from **1 to 2 mL human plasma**. The table below shows the number of multi-well plates needed for processing of plasma volumes from 0.5–2 mL.

Plasma volume [mL]	Amount of samples	Number of 96-Square-well blocks	Number of 24-Square-well blocks
0.5	96	1	-
1	24	-	1
	48	1	2
	96	2	4
2	24	1	1
	48	2	2
	96	4	4

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · Fax: +49 24 21 969-199 · tech-bio@mn-net.com · ***www.mn-net.com***

Processing of 1 to 2 mL plasma using 96-well plates

The procedure below describes the isolation of cell-free DNA from 1 mL or 2 mL human plasma using 96-well plates. Samples have to be divided equally into two or four 96-Square-well Blocks.

1 Split sample

Add **12.5 µL Liquid Proteinase K** into each well of both 96-Square-well Blocks

Split samples into aliquots of 500 µL into two or four 96-Square-well Blocks and mix by pipetting.

Add **200 µL Buffer PML** and mix by pipetting.

Incubate at **56 °C** for **30 min** (ideally with shaking). For Cell-Free DNA BCT® (Streck), incubate 60 min; ideally with shaking.

Insert spacers 'MTP/MULTI-96 PLATE', the Waste Container and the Wash Plate into the manifold base. Place the manifold lid on top and then the **NucleoSpin® 96 Plasma Binding Plate**

While incubating the lysis, apply **400 µL Buffer PMA** to the columns. Incubate one minute, then apply vacuum of **-0.4 bar*** for 1 min.

2 Adjust binding conditions

Add **1 mL Buffer PMB** to each well and mix by pipetting.

3 Bind DNA

Apply prepared lysates to the NucleoSpin® 96 Plasma Binding Plate in aliquots of 1 mL.

Lysates can be loaded continuously while they are passing the membrane. Remove, empty and replace Waste Container after the third loading step as well as after column loading is completed.

Continue with step 4 of the standard protocol.

* Reduction of atmospheric pressure

Processing of 2 mL plasma using 24-well plates

The procedure below describes the isolation of cell-free DNA from **2 mL human EDTA plasma** using 24-Square-well blocks. (Four 24-Square-well blocks are needed for 96 samples).

1 Lyse sample

Add **50 µL Liquid Proteinase K** into each well of a 24-Square-well block.

Add **2 mL** plasma and mix by pipetting.

Add **800 µL Buffer PML** and mix by pipetting.

Incubate at **56 °C** for **30 min** (ideally with shaking). For Cell-Free DNA BCT® (Streck), incubate 60 min; ideally with shaking.

Insert spacers 'MTP/MULTI-96 PLATE', the Waste Container and the Wash Plate into the manifold base. Place the manifold lid on top and then the **NucleoSpin® 96 Plasma Binding Plate**.

While incubating the lysis, apply **400 µL Buffer PMA** to the columns. Incubate one minute, then apply vacuum of **-0.4 bar*** for **1 min**.

2 Adjust binding conditions

Add **4 mL Buffer PMB** to each well and mix by pipetting.

3 Bind DNA

Apply prepared lysates to the NucleoSpin® 96 Plasma Binding Plate in aliquots of 1 mL.

Lysates can be loaded continuously while they are passing the membrane. Remove, empty and replace Waste Container after the third loading step as well as after column loading is completed.

Continue with step 4 of the standard protocol.

* Reduction of atmospheric pressure