Supplementary protocol

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NucleoSpin® Gel and PCR Clean-up – PCR Clean-up 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****.*

The following protocol is suitable for PCR Clean-up as well as DNA concentration and removal of salts, enzymes, etc. from enzymatic reactions (SDS < 0.1 %). Instead of centrifugal forces, vacuum is used to process the samples eliminating the need of removing and emptying the Collection Tubes after each binding and washing step.

Any vacuum manifold with Luer-connections is suitable, using the NucleoVac 24 Vacuum Manifold is recommended (see ordering information).

1 Adjust DNA binding conditions

+ 2 vol NTI per 1 vol sample

For very small sample volumes < 30 µL adjust the volume of the reaction mixture to 50–100 µL with water.

It is not necessary to remove mineral oil.

Mix **1 volume of sample** with **2 volumes** of **Buffer NTI** (e.g., mix 100 µL PCR reaction and 200 µL Buffer NTI).

Note: For removal of small fragments like primer dimers dilutions of Buffer NTI can be used instead of 100 % Buffer NTI. Please refer to section 2.3 of the NucleoSpin® Gel and PCR Clean-up manual.

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2 Bind DNA

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

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

When the sample has passed the NucleoSpin® Gel and PCR Clean-up Column, release the vacuum.

If necessary load remaining sample and repeat the step.

Load sample

-0.2 to -0.4 bar*

1 min

3 Wash silica membrane

Add **700 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Column. Do not close the lid!

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

When the sample has passed the NucleoSpin® Gel and PCR Clean-up Column, release the vacuum.

+ 700 µL NT3

-0.2 to -0.4 bar*

1 min

4 <u>Optional:</u> Repeat washing step

Repeat previous washing step to minimize chaotropic salt carry-over and to improve A_{260}/A_{230} values (see section 2.7 of the NucleoSpin® Gel and PCR Clean-up manual).

+ 700 µL NT3

-0.2 to -0.4 bar*

1 min

* Reduction of atmospheric pressure

5 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 NT3** completely. Run the vacuum pump continuously. Achieving and keeping a continuous air flow is of more importance than reaching the precise mentioned reduction of atmospheric pressure. Do not close the lid!

Release the vacuum.

Option 2: Drying by centrifugation

11,000 x *g*

1 min

Place the NucleoSpin® Gel and PCR Clean-up Column into a Collection Tube (2 mL). Centrifuge for **1 min** at **11,000 x *g*** to remove Buffer NT3 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 NT3 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.

6 Elute DNA

+ 15–30 µL NE

RT

1 min

11,000 x *g*

1 min

Place the NucleoSpin® Gel and PCR Clean-up Column into a new 1.5 mL microcentrifuge tube (not provided). Add **15–30 µL Buffer NE** and incubate at room temperature (18–25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 x *g***.

Note: DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with either fresh Buffer NE (reducing concentration), or by reloading the eluates for a second elution step as well as by increased incubation times at elevated temperatures (e.g., 70 °C).

* Reduction of atmospheric pressure

Supplementary protocol

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NucleoSpin® Gel and PCR Clean-up – DNA extraction from agarose gels using a vacuum manifold (Rev. 01, October 2016)

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1 Excise DNA fragment / solubilize gel slice

Note: Minimize UV exposure time to avoid damaging the DNA. Refer to section 2.5 for more tips on gel extraction.

+ 200 µL NTI per 100 mg gel

50 °C

5–10 min

Take a clean scalpel to excise the DNA fragment from an agarose gel. Remove all excess agarose.

Determine the weight of the gel slice and transfer it to a clean tube.

For each 100 mg of agarose gel < 2 % add 200 µL Buffer NTI (e.g., mix 200 mg of a 1 % agarose gel slice and 400 µL Buffer NTI).

For gels containing > 2 % agarose, double the volume of Buffer NTI.

Incubate the sample at 50 °C until the gel slice is completely dissolved. Vortex the sample briefly every 2–3 min.

2 Bind DNA

Load sample

-0.2 to -0.4 bar*

1 min

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

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

When the sample has passed the NucleoSpin® Gel and PCR Clean-up Column, release the vacuum.

If necessary, load remaining sample and repeat the step.

* Reduction of atmospheric pressure

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3 Wash silica membrane

Add **700 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Column. Do not close the lid!

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

When the sample has passed the NucleoSpin® Gel and PCR Clean-up Column, release the vacuum.

+ 700 µL NT3
-0.2 to -0.4 bar*
1 min

4 <u>Optional:</u> Repeat washing step

Repeat previous washing step to minimize chaotropic salt carry-over and to improve A_{260}/A_{230} values (see section 2.7 of the NucleoSpin® Gel and PCR Clean-up manual).

+ 700 µL NT3
-0.2 to -0.4 bar*
1 min

5 Dry silica membrane

<u>Option 1:</u> Drying by vacuum

Apply vacuum of **-0.4 to -0.6 bar*** for **5 min** to remove **Buffer NT3** completely. Run the vacuum pump continuously. Achieving and keeping a continuous air flow is of more importance than reaching the precise mentioned reduction of atmospheric pressure. Do not close the lid!

Release the vacuum.

-0.4 to -0.6 bar*
5 min

<u>Option 2:</u> Drying by centrifugation

Place the NucleoSpin® Gel and PCR Clean-up Column into a Collection Tube (2 mL). Centrifuge for **1 min** at **11,000 x *g*** to remove Buffer NT3 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.

<u>Note:</u> Residual ethanol from Buffer NT3 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

11,000 x *g*
1 min

* Reduction of atmospheric pressure

6 Elute DNA

Place the NucleoSpin® Gel and PCR Clean-up Column into a new 1.5 mL microcentrifuge tube (not provided). Add **15–30 µL Buffer NE** and incubate at room temperature (18–25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 x *g***.

Note: DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with either fresh Buffer NE (reducing concentration), or by reloading the eluates for a second elution step as well as by increased incubation times at elevated temperatures (e.g., 70 °C).

+ 15–30 µL NE

RT

1 min

11,000 x *g*

1 min