

Plasmid DNA concentration and desalting

User manual

NucleoBond[®] Finalizer
NucleoBond[®] Finalizer Large

October 2015 / Rev. 07

Plasmid DNA concentration and desalting

Protocol at a glance (Rev. 07)

	NucleoBond® Finalizer	NucleoBond® Finalizer Large
1 Precipitate DNA	3.5 mL isopropanol for 5 mL eluate Mix thoroughly (Watch salt concentration) RT, 2 min	10.5 mL isopropanol for 15 mL eluate Mix thoroughly (Watch salt concentration) RT, 2 min
2 Load precipitate	 Press slowly!	
3 Wash precipitate	2 mL 70 % ethanol	5 mL 70% ethanol
4 Dry filter membrane	≥ 3 x air	≥ 6 x air
5 Elute DNA	Two-fold elution: 1st elution 200–800 µL Tris or TE buffer 2nd elution Load first eluate completely Recover as much eluate as possible by quickly pressing air through the filter.	Two-fold elution: 1st elution 400–1000 µL Tris or TE buffer 2nd elution Load first eluate completely

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1 Components

1.1 Kit contents

	NucleoBond® Finalizer	NucleoBond® Finalizer Plus
REF	740519.20	740520.20
NucleoBond® Finalizer	20	20
30 mL Syringes	2	20
1 mL Syringes	2	20
User manual	1	1

	NucleoBond® Finalizer Large	NucleoBond® Finalizer Large Plus
REF	740418.20	740419.20
NucleoBond® Finalizer Large	20	20
30 mL Syringes	2	20
1 mL Syringes	2	20
User manual	1	1

1.2 Reagents to be supplied by the user

- Isopropanol (room-temperated)
- 70 % ethanol (room-temperated)
- Buffer for reconstitution of DNA (e.g., Tris or TE buffer; see section 2.4)

1.3 About this user manual

It is strongly recommended that first time users of the NucleoBond® Finalizer (Large) read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at www.mn-net.com.

2 Product description

2.1 The basic principle

NucleoBond® Finalizers are designed for quick concentration and desalination of plasmid and cosmid DNA eluates that are obtained by anion-exchange chromatographic DNA purification with **NucleoBond® AX** and **NucleoBond® Xtra Columns**. The sample is precipitated with isopropanol and loaded onto a **NucleoBond® Finalizer** membrane by means of a syringe. After an ethanolic washing step and drying of the membrane the pure DNA can be eluted with low salt buffer for further use. The **NucleoBond® Finalizer** technology replaces the tedious and time consuming centrifugation step which hitherto follows every isopropanol precipitation of diluted DNA samples. Furthermore problems like lost DNA pellets or incomplete solubilization of hardly visible precipitates can easily be circumvented. The pure concentrated and salt free DNA can be used directly for all common downstream applications like transfection, in vitro transcription, automated or manual sequencing, cloning, hybridization, and PCR.

2.2 Kit specifications

- The **NucleoBond® Finalizer** kits contain the **NucleoBond® Finalizers** and a set of 30 mL and 1 mL syringes for sample loading and DNA elution.
- The protocol is suitable for purifying most plasmids and cosmids ranging from 2–50 kbp.
- **NucleoBond® Finalizers** are polypropylene filters containing a special silica membrane. The **NucleoBond® Finalizer** with a binding capacity of 500 µg is suitable for use with **NucleoBond® PC 100**, **PC 500**, and **NucleoBond® Xtra Midi** kits. The **NucleoBond® Finalizer Large** can hold up to 2000 µg and is suitable for use with **NucleoBond® Xtra Maxi** and **NucleoBond® PC 2000** kits. Both **NucleoBond® Finalizers** are also suitable for all other plasmid DNA purification or concentration procedures resulting in an isopropanol DNA precipitate.
- The **NucleoBond® Finalizers** are free of endotoxins and can therefore be used with **NucleoBond® PC EF** kits and **NucleoBond® Xtra EF** kits as well.
- All **NucleoBond® Finalizers** are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of DNase and RNase.
- Using the **NucleoBond® Finalizer** kits reduces the necessary hands-on time to obtain concentrated DNA from over an hour to about 5 minutes.
- Due to the small filter size an effective washing step reduces chloride concentration in the final eluate to < 0.3 µg/µL.
- The supplied syringes may be reused when washed with sufficient water.

Table 1: Kit specifications at a glance

Parameter	NucleoBond® Finalizer	NucleoBond® Finalizer Large
Loadable volume	Unlimited	
Binding capacity	500 µg DNA	2000 µg DNA
Elution volume	200–800 µL	400–1000 µL
Typical recovery (elution volume dependent)	60–90 %	
Typical concentration (elution volume dependent)	0.1–3 µg/µL	
Plasmid size	2–50 kbp	
Residual chloride concentration	< 0.3 µg/µL	
Dead volume	~ 30 µL	~ 60 µL
Preparation time	5 min	
To be combined with	NucleoBond® Xtra Midi NucleoBond® Xtra Midi EF NucleoBond® PC 100 NucleoBond® PC 500 NucleoBond® PC 500 EF	NucleoBond® Xtra Maxi NucleoBond® Xtra Maxi EF NucleoBond® PC 2000 NucleoBond® PC 2000 EF

2.3 DNA precipitation

In general diluted DNA can be precipitated by adding 0.7 volumes of isopropanol to solutions containing at least 0.3 M sodium acetate, 0.2 M sodium chloride, or 0.2 M potassium chloride. Make sure your samples contain enough salt before using the **NucleoBond® Finalizer** kits.

NucleoBond® PC 100, PC 500, PC 2000, Xtra Midi, and Xtra Maxi eluates as well as similar anion-exchange chromatography eluates already contain a sufficient amount of salt to allow a quantitative DNA precipitation. Here, only addition of 0.7 volumes of **room temperature (20–25 °C) isopropanol** (not provided in the kit) is necessary. Do not let the plasmid DNA solution drop into a vial with isopropanol, because this might lead to spontaneous co-precipitation of salt.

2.4 Elution procedure

Elution from the **NucleoBond® Finalizers** is carried out with slightly alkaline buffers like TE buffer (10 mM Tris/HCl, pH 7.5, 1 mM EDTA) or Tris buffer (5 mM Tris/HCl, pH 8.5). DNA precipitation is performed at room temperature in contrast to a standard incubation on ice or -20 °C in order to avoid co-precipitation of salt.

For maximum yield it is recommended to perform the elution step twice. The first elution step is done using fresh buffer whereas in the second elution step the eluate from the first elution is reapplied on the **NucleoBond® Finalizer** to allow complete solubilization of the plasmid.

DNA recovery highly depends on the used elution buffer volume. Large volumes result in a high recovery of up to 90 % but in a lower DNA concentration. Small elution volumes on the other hand increase the concentration but at the cost of DNA yield.

If a small volume is chosen, make sure to recover as much eluate as possible from the syringe and **NucleoBond® Finalizer** by pressing air through the **NucleoBond® Finalizer** several times after elution and collecting every single droplet to minimize the dead volume.

Figure 1 and Figure 2 on 7 and 8 illustrate exemplarily how DNA recovery and final DNA concentration depend on the buffer volume which is used for elution of DNA from the **NucleoBond® Finalizer** and **NucleoBond® Finalizer Large**, respectively.

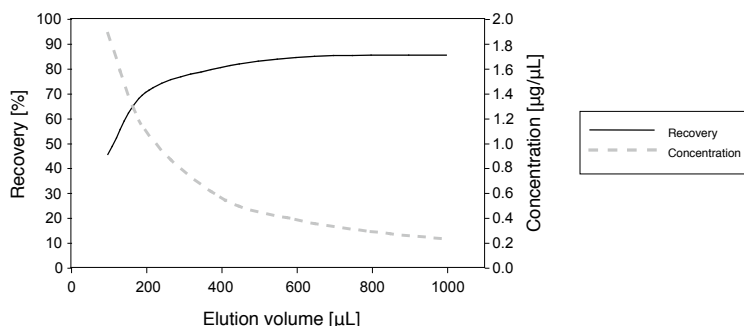


Figure 1 Final DNA recovery and concentration after NucleoBond® Finalizer application

A NucleoBond® Xtra Midi eluate containing 250 µg plasmid DNA (8 kbp) was loaded onto a NucleoBond® Finalizer and eluted two-fold with increasing volumes of TE buffer.

The **NucleoBond® Finalizer** is designed to hold a maximum of 500 µg DNA and is therefore ideally suited to be used in combination with **NucleoBond® PC 100**, **PC 500**, and **NucleoBond® Xtra Midi**. Maximum DNA recovery can be achieved by using > 600 µL of elution buffer. For a higher concentration experienced users can lower the elution buffer volume to 400–200 µL.

Table 2 gives an overview about recovery and concentration of different amounts of plasmid DNA loaded onto a **NucleoBond® Finalizer** and eluted two-fold with increasing

volumes of TE buffer. Please refer to this table to select an elution buffer volume that meets your needs best.

Table 2: DNA recovery and concentration for the NucleoBond® Finalizer

Loaded DNA	Elution volume					
	100 µL	200 µL	400 µL	600 µL	800 µL	1000 µL
500 µg	35 %	60 %	70 %	75 %	75 %	75 %
	2.5 µg/µL	2.3 µg/µL	1.2 µg/µL	0.8 µg/µL	0.6 µg/µL	0.5 µg/µL
250 µg	40 %	65 %	75 %	80 %	80 %	80 %
	1.9 µg/µL	1.1 µg/µL	0.6 µg/µL	0.4 µg/µL	0.3 µg/µL	0.2 µg/µL
100 µg	45 %	70 %	80 %	85 %	85 %	85 %
	0.7 µg/µL	0.4 µg/µL	0.2 µg/µL	0.1 µg/µL	0.1 µg/µL	0.1 µg/µL
50 µg	30 %	75 %	85 %	90 %	90 %	90 %
	0.3 µg/µL	0.2 µg/µL	0.1 µg/µL	0.1 µg/µL	0.1 µg/µL	< 0.1 µg/µL

DNA recovery DNA concentration

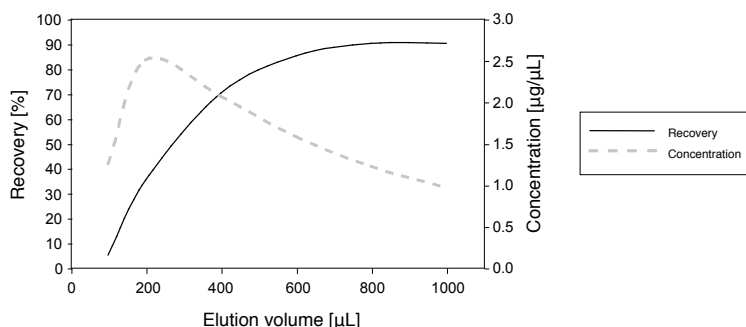


Figure 2 Final DNA recovery and concentration after NucleoBond® Finalizer Large application

A NucleoBond® Xtra Maxi eluate containing 1000 µg plasmid DNA (8 kbp) was loaded onto a NucleoBond® Finalizer Large and eluted two-fold with increasing volumes of TE buffer.

NucleoBond® Xtra Maxi and **NucleoBond® PC 2000** eluates are easily concentrated with a **NucleoBond® Finalizer Large** which is able to bind up to 2000 µg plasmid DNA. Maximum DNA recovery can be achieved by using > 800 µL of elution buffer. For a higher concentration experienced users can lower the elution buffer volume to 600–400 µL.

Table 3 gives an overview about recovery and concentration of different amounts of plasmid DNA loaded onto a **NucleoBond® Finalizer Large** and eluted two-fold with

increasing volumes of TE buffer. Please refer to this tables to select an elution buffer volume that meets your needs best.

Table 3: DNA recovery and concentration for the NucleoBond® Finalizer Large

Loaded DNA	Elution volume					
	100 µL	200 µL	400 µL	600 µL	800 µL	1000 µL
1500 µg	5 %	30 %	65 %	80 %	85 %	90 %
	1.9 µg/µL	3.2 µg/µL	2.9 µg/µL	2.2 µg/µL	1.7 µg / µL	1.4 µg/µL
1000 µg	5 %	35 %	70 %	85 %	90 %	90 %
	1.3 µg/µL	2.5 µg/µL	2.1 µg/µL	1.6 µg/µL	1.2 µg / µL	1.0 µg/µL
500 µg	10 %	40 %	70 %	85 %	85 %	90 %
	1.3 µg/µL	1.4 µg/µL	1.0 µg/µL	0.8 µg/µL	0.6 µg / µL	0.5 µg/µL
100 µg	15 %	45 %	70 %	80 %	85 %	90 %
	0.4 µg/µL	0.3 µg/µL	0.2 µg/µL	0.1 µg/µL	0.1 µg / µL	1.0 µg/µL

DNA recovery DNA concentration

3 Storage conditions

All kit components can be stored at room temperature (18–25 °C).

4 Safety instructions

The components of the NucleoBond® Finalizer and NucleoBond® Finalizer Large kits do not contain hazardous contents.

5 Protocol for DNA concentration and desalination

NucleoBond® Finalizer

NucleoBond® Finalizer Large

1 Precipitate DNA

Note: Check DNA concentration photometrically before precipitation. This helps to choose the best buffer volume in step 5 and allows calculation of the recovery after concentration.

Using eluates from anion-exchange chromatography add **0.7 volumes** of **room-temperature isopropanol** (not supplied with the kit). **Vortex well** and let the mixture sit for **2 minutes**.

(E.g., for 5 mL NucleoBond® PC 100 or Xtra Midi eluate add **3.5 mL** isopropanol, for 15 mL NucleoBond® PC 500 or Xtra Maxi eluate add **10.5 mL** isopropanol.)

If your sample does **not** contain high salt concentrations (e.g., 0.2 M sodium chloride, 0.3 M sodium acetate, or 0.2 M potassium chloride), add about 1/10 volume of 3 M sodium acetate (pH 4.2) before adding isopropanol.

3.5 mL for 5 mL eluate

10.5 mL for 15 mL eluate

2 Load precipitate

Remove the plunger from a **30 mL Syringe** and attach a NucleoBond® Finalizer to the outlet.

Fill the precipitation mixture into the syringe, insert the plunger, hold the syringe in a vertical position, and press the mixture **slowly** through the NucleoBond® Finalizer using **minimal force**. Discard the flowthrough.

3 Wash precipitate

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

Fill **70 % ethanol** (not supplied with the kit) into the syringe, insert the plunger, hold the syringe in vertical position, and press the ethanol **slowly** through the NucleoBond® Finalizer. Discard the ethanol.

2 mL

5 mL

NucleoBond® Finalizer**NucleoBond® Finalizer Large**

4 Dry filter membrane

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer. Press air through the NucleoBond® Finalizer while **touching a tissue as strongly as possible** with the tip of the NucleoBond® Finalizer to soak up ethanol.

Repeat this step at least as often as indicated below until no more ethanol leaks from the NucleoBond® Finalizer.

Note: *A new dry syringe can be used to speed up the procedure (not provided).*

≥ 3 times until dry

≥ 6 times until dry

Optional: You can incubate the NucleoBond® Finalizer for 10 minutes at 80 °C to minimize ethanol carry-over. However, the final recovery may be reduced by over-drying the DNA.

5 Elute DNA

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger of a **1 mL Syringe** and attach the NucleoBond® Finalizer to the syringe outlet.

Note: *Refer to section 2.4, Table 2 (Midi) or Table 3 (Maxi) to choose the appropriate volume of elution buffer.*

Pipette an appropriate volume of Tris buffer or TE buffer (see section 2.4) into the syringe. Do not use pure water unless pH is definitely higher than 7. Place the NucleoBond® Finalizer outlet in a vertical position over a fresh collection tube (not provided) and **elute plasmid DNA very slowly**, drop by drop, by inserting the plunger.

200–800 µL

400–1000 µL

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

! Transfer the first eluate back into the syringe and elute into the same collection tube a second time.

Load first eluate completely

Load first eluate completely

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger to aspirate air, reattach the NucleoBond® Finalizer, and **press the air out again to force out as much eluate as possible**.

Determine plasmid yield by UV spectroscopy and confirm plasmid integrity by agarose gel electrophoresis (see section 6.1).

6 Appendix

6.1 Determination of DNA yield and quality

The yield of a plasmid preparation should be estimated prior to and after the isopropanol precipitation in order to calculate the recovery after precipitation and to find the best elution volume for the NucleoBond® Finalizers. Simply use the NucleoBond® PC or NucleoBond® Xtra elution buffer (for estimation prior to precipitation) or the respective low salt buffer (for estimation after the precipitation) as a blank in your photometric measurement.

The nucleic acid **concentration** of the sample can be calculated from its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µg DNA/mL. Note that the absolute measured absorbance should lie between 0.1 and 0.7 to be in the linear part of Lambert-Beer's law. Dilute your sample in the respective buffer if necessary.

The plasmid **purity** can be checked by UV spectroscopy as well. A ratio of A_{260}/A_{280} between 1.80–1.90 and A_{260}/A_{230} around 2.0 indicates pure plasmid DNA. An A_{260}/A_{280} ratio above 2.0 is a sign for too much RNA in your preparation, an A_{260}/A_{280} ratio below 1.8 indicates protein contamination.

Plasmid **quality** can be checked by running the precipitated samples on a 1 % agarose gel. This will give information on conformation and structural integrity of isolated plasmid DNA i.e. it shows whether the sample is predominantly in the favorable super-coiled (ccc, usually the fastest band), open circle (oc) or even linear form.

6.2 Troubleshooting

If you experience problems with reduced purity or quality, we recommend to check your DNA before concentration with the **NucleoBond® Finalizers**. Usually the purification procedure itself and not the **NucleoBond® Finalizer** is causing these problems.

However, if your main difficulty is low yield although you had plenty of DNA before applying the **NucleoBond® Finalizer** then please check the following table for detailed explanations.

Problem	Possible cause and suggestions
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Incomplete precipitation

- Usually all sizes of plasmid or cosmid DNA are precipitated with high efficiency of about 90 % at room temperature in the presence of at least 0.2 M sodium chloride or 0.3 M sodium acetate and 40 % isopropanol. Therefore make sure your sample contains enough salt and alcohol. Almost 100 % recovery can be achieved by incubation at 0 °C or -20 °C for 20 min. But this may lead to co-precipitation of salt and clogging of the NucleoBond® Finalizer membrane and is therefore not recommended.

Plasmid size

- Precipitation efficiency is almost independent of plasmid size, but elution from the NucleoBond® Finalizers becomes more and more difficult with increasing construct size. If you face low yields with large cosmids you may try heating the NucleoBond® Finalizer, the syringes, and elution buffer to 70 °C.

Dead volume too high

No or low
plasmid DNA
yield

- If high concentration of plasmid DNA is the main aim, elution should be performed in small volumes. Naturally a portion of the eluate will be lost in the syringe and on the NucleoBond® Finalizer. To minimize these losses in the second elution step, try to transfer even the last droplet from the syringe to the NucleoBond® Finalizer, for example by tapping the NucleoBond® Finalizer and syringe onto the bench top. Then fill the syringe with air and press forcefully the last droplets out of the NucleoBond® Finalizer. Repeat this step several times. You might have to practice this procedure several times to achieve optimal results. An acceptable dead volume is smaller than 30 µL with NucleoBond® Finalizer and 60 µL with NucleoBond® Finalizer Large.

Elution volume too small

- Since there are certain dead volumes of about 30 µL (NucleoBond® Finalizer) and 60 µL (NucleoBond® Finalizer Large), reasonable elution volumes start with 200 µL (NucleoBond® Finalizer) and 400 µL (NucleoBond® Finalizer Large), respectively. Furthermore smaller volumes are insufficient to wet the entire membrane and will drastically decrease your yield. Refer to section 2.4, Table 2 (NucleoBond® Finalizer) and Table 3 (NucleoBond® Finalizer Large) to estimate the recovery that can be expected depending on elution buffer volume.

Problem	Possible cause and suggestions
No or low plasmid DNA yield (continued)	<i>Elution too fast</i> Plasmid DNA needs time to dissolve. Elute really very slowly, drop by drop. Repeat the elution procedure using the first eluate.
	<i>Forgot to elute a second time</i> Repeating the elution procedure with the first eluate is crucial for optimal yields. However, eluting a third time shows no more improvement.
Low DNA concentration	<i>Low overall yield</i> Refer to detailed troubleshooting “No or low plasmid DNA yield” and lower your elution buffer volume. Refer to section 2.4, Table 2 and Table 3 to estimate the DNA concentrations that can be expected.
	<i>Fresh elution buffer used for second elution step</i> The second elution step is crucial for optimal yield but to achieve a high DNA concentration the eluate of the first elution step has to be used for the second elution.
	<i>Not enough DNA loaded</i> Since there is a technical limitation to at least 200 µL (NucleoBond® Finalizer) and 400 µL (NucleoBond® Finalizer Large) of elution buffer due to membrane wetting and dead volume, a minimal amount of DNA has to be loaded to achieve a desired concentration. If possible try to pool several DNA precipitation batches since percentage of recovery and concentration significantly increase with higher amounts of loaded DNA (see section 2.4, Table 2 and Table 3).

Problem	Possible cause and suggestions
Low recovery when using the Finalizer	Depending on the total amount of the precipitated plasmid it will need some time to redissolve completely. Resuspension time might be too short for a complete recovery, if the redissolving buffer is passed by the precipitated plasmid on the Finalizer membrane too quickly. If a high recovery is mandatory it is recommended to incubate the precipitated plasmid in the redissolving buffer during the elution step. Therefore, do not press the elution buffer through the Finalizer in one rush, but rather stop pressing the buffer through as soon as the first drops have passed the outlet. Let the redissolving buffer sit on the membrane in the Finalizer for 5 minutes at room temperature before completing the elution step. Reload the eluate onto the Finalizer and repeat the procedure at least once. General recommendations are also valid here: push the redissolving buffer through the membrane slowly, increase the elution volume to gain a higher recovery and blow out the dead volume completely by pushing air through the Finalizer.

6.3 Ordering information

Product	REF	Pack of
NucleoBond® Finalizer (for use with NucleoBond® Xtra Midi, Midi EF, NucleoBond® PC 100, PC 500, PC 500 EF)	740519.20	20 filters 2 syringe sets
NucleoBond® Finalizer Plus (for use with NucleoBond® Xtra Midi, Midi EF, NucleoBond® PC 100, PC 500, PC 500 EF)	740520.20	20 filters 20 syringe sets
NucleoBond® Finalizer Large (for use with NucleoBond® Xtra Maxi, Maxi EF, NucleoBond® PC 2000, PC 2000 EF)	740418.20	20 large filters 2 syringe sets
NucleoBond® Finalizer Large Plus (for use with NucleoBond® Xtra Maxi, Maxi EF, NucleoBond® PC 2000, PC 2000 EF)	740419.20	20 large filters 20 syringe sets
NucleoBond® Xtra Midi	740410.10 / .50 / .100	10 / 50 / 100 preps
NucleoBond® Xtra Midi Plus (including NucleoBond® Finalizer)	740412.10 / .50	10 / 50 preps
NucleoBond® Xtra Maxi	740414.10 / .50 / .100	10 / 50 / 100 preps
NucleoBond® Xtra Maxi Plus (including NucleoBond® Finalizer Large)	740416.10 / .50	10 / 50 preps
NucleoBond® PC 100	740573 / .100	20 / 100 preps
NucleoBond® PC 500	740574 / .25 / .50 / .100	10 / 25 / 50 / 100 preps
NucleoBond® PC 2000	740576	5 preps

Visit **www.mn-net.com** for more detailed product information.

6.4 Product use restriction/warranty

NucleoBond® Finalizer kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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