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Plasmid DNA Concentration and Desalination

User Manual

NucleoBond® Finalizer

NucleoBond® Finalizer Large

August 2009 / Rev. 05

MACHEREY-NAGEL

Plasmid DNA Concentration and Desalination

Protocol-at-a-glance (Rev. 05)

		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	 Press fast!	≥ 6 x air
5	**Elute DNA**	Two-fold elution: **1st elution** 200 - 800 µl Tris or TE buffer **2nd elution** Load first eluate completely	 Press very slowly!	Two-fold elution: **1st elution** 400 - 1000 µl Tris or TE buffer **2nd elution** Load first eluate completely
		Recover as much eluate as possible by quickly pressing air through the filter.		

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1 Components

1.1 Kit contents

	NucleoBond® Finalizer	NucleoBond® Finalizer Plus
Cat. No.	**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
Cat. No.	**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-temperatured)
- 70% ethanol (room-temperatured)
- 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 µl	> 400 µl
Typical recovery (elution volume dependent)	40 - 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
Time/prep	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.

Figures 1 and 2 on page 9 and 10 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.

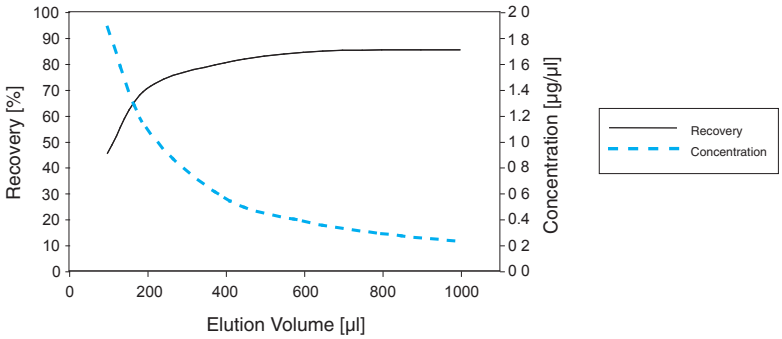

Figure 1 Final DNA recovery and concentration after NucleoBond® Finalizer application

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

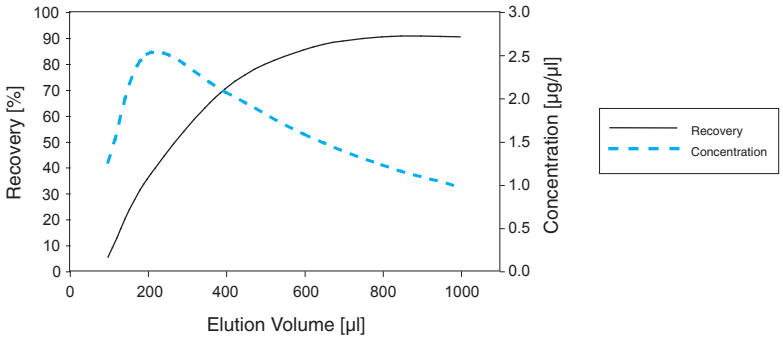

Figure 2 Final DNA recovery and concentration after NucleoBond® Finalizer Large application

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%**	**90%**	**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	0.1 µg/µl

DNA recovery
DNA concentration

3 Storage conditions

All kit components can be stored at room temperature (20 - 25°C).

4 Safety instructions – risk and safety phrases

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: The **NucleoBond® Finalizer** only holds up to **500 µg** and the **NucleoBond® Finalizer Large** is limited to **2000 µg** of plasmid DNA. Loading more DNA might lead to clogging and complete loss of your sample. Thus, it is highly recommended to determine plasmid yield by measuring A_{260} before precipitating the DNA (see section 6.1). Furthermore, 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)

Note: 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 **constant force**. Discard the flow-through.

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	≥ 6 times

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 1 (Midi) or 2 (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. Place the NucleoBond® Finalizer outlet in a vertical position over a fresh collection tube and **elute plasmid DNA** dropwise 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**.

NucleoBond® Finalizer	NucleoBond® Finalizer Large

6 Determination of yield

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 supercoiled (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
No or low plasmid DNA yield	*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 -20C 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* • Especially when you aim for high concentration you need to elute in small volumes. But naturally you will lose parts of your eluate 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, e.g. by tapping the NucleoBond® Finalizer and syringe onto the benchtop. 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 the NucleoBond® Finalizer and 60 µl with the NucleoBond® Finalizer Large. *Elution volume too small* • Since there is a certain dead volume 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 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)*	*Forgot to elute a second time* • Repeating the elution procedure with the first eluate is crucial for optimal yields. Eluting a third time shows no more improvement.
Low DNA concentration	*Low overall yield* • Refer to problem “No or low plasmid DNA yield” in this table and lower your elution buffer volume. Refer to section 2.4, Table 2 and 3 to estimate the DNA concentrations that can be expected. *Fresh elution buffer used for second elution step* • 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 reused for the second elution. *Not enough DNA loaded* • 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, Tables 2 and 3).

6.3 Ordering information

Product	Cat. No.	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 were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY.** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoBond® Finalizer** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

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Last updated: 12/2006, Rev. 02