NUCLEOCEL *DELTA*

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column.
NUCLEOCEL *DELTA*, *DELTA S*, *DELTA-RP* or *DELTA-RP S* columns are quality products based on robust silica. The silica is covered with a cellulose derivative as chiral selector. Several solvents which are common in HPLC or SPE may destroy the column by dissolving the chiral selector. Consequently, prior to column installation, you should familiarize yourself with the contents of this instruction leaflet. If carefully and properly used excellent chromatographic results and long column lifetime can be achieved. This column has specifically been developed for the chromatographic separation of optical isomers and demonstrated to be a successful tool for optical resolution as well as for the determination of the enantiomeric purity. All HPLC columns must exclusively be used in accordance with universally accepted laboratory regulations and HPLC working methods. Before running the column the entire analytical system (column and equipment) has to be carefully checked by the operator. Chromatographic conditions (mobile phase, flow, temperature etc.) must be adapted to the analytical task. MACHEREY-NAGEL does not give any warranty and is not liable for the success of a separation or application. If you have any questions after reading this leaflet, please call our service / technical support.

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Safety indication
Follow the general safety instructions for handling of HPLC solvents used as mobile phases (e.g., *n*-heptane, propanol, acetonitrile) and take precautions against any kind of injuries or damage to health (e.g., skin and eye protection in case of broken capillaries). Disposal of used HPLC columns must follow international, national and local environmental protection regulations. The use of HPLC columns is only permitted to staff members, who are qualified in their field. Keep HPLC columns away from children. MACHEREY-NAGEL disclaims and excludes all warranties of any kind or nature whatsoever and MN shall not be liable for any damages (whether direct, indirect, foreseeable, incidental, compensatory, consequential or special), whether based upon warranty, contract, tort or strict liability, if damages and / or losses occur caused by improper use, maintenance, neglect or improper treatment (especially opening of the column and exposure of the column bed).

Description of the column
The stationary phase of the NUCLEOCEL *DELTA* columns is a spherical silica covered with cellulose tris-(3,5-dimethylphenylcarbamate). Chiral recognition is based on the selective interaction between the cellulose derivative and the analytes. Also hydrophobic interaction as well as interaction from polar groups and sterical effects influence the separation mechanism. The column has proven useful for chiral analysis of pharmaceutically active compounds, chiral pollutants (e.g., herbicides, PCBs), chiral compounds in food (dyes, preservatives), chiral catalysts and bioorganic compounds. Application notes can be found in our application database on the internet under ***www.mn-net.com/apps***.

Installation
Before an installation of NP columns, flush your whole HPLC system including syringe and sample loop with *n*-heptane – 2-propanol (90:10, v/v) or the RP columns with acetonitrile – water (50:50, v/v). The columns should be installed in the flow direction indicated on the column label. They are connected with 1/16" capillaries and fittings, typical for HPLC instruments.

Guard columns
For protection and an extension of column lifetime the column should always be used with a guard column. The filter elements and the adsorbent in the guard column retain contaminants from the sample or the eluent. Connection of the guard column with the separation column is made by a suitable guard column holder (see ***www.mn-net.com*** or the MN chromatography catalog). Cartridge replacement is required when increased column pressure and / or loss of performance is observed.

Sample
Generally, the sample is dissolved in the eluent. Before the injection of the sample onto the column the sample should be passed through a syringe filter (e.g., CHROMAFIL® Xtra PET, 0.45 µm, 25 mm, REF 729220). Never use solvents for samples, which damage the column (see eluent)! If injected samples are still turbid even after filtration, the lifetime of the column may be significantly reduced. The sample volume should be as small as possible to achieve an optimal resolution.

Eluent
Please note! Never use the following solvents neither as mobile phase nor as solvent for your samples: Ethers including tetrahydrofurane and dioxane, halogenated hydrocarbons (e.g., dichloromethane, chloroform), ketones (e.g., acetone, propanone, 2-butanone), ethylacetate, aromatic hydrocarbons like toluene or xylene, dimethylsulfoxide, dimethylformamide, dimethylacetamide
NP columns: Eluent in the column is *n*-heptane – 2-propanol (90:10, v/v). As mobile phases in normal phase mode (NP) *n*-heptane – 2-propanol or *n*-heptane – ethanol mixtures are used. Instead of *n*-heptane, *n*-hexane can be used. For the analysis of basic compounds a possible tailing can be reduced by the addition of 0.1 % diethylamine and for acidic compounds by 0.1 % trifluoroacetic acid (TFA) or acetic acid. Eluents should always be filtered through a 0.2–0.45 µm membrane filter and degassed.
If you want to work under RP conditions we recommend to use *DELTA-RP* columns which already contained an RP eluent (see RP columns). Although it is possible to switch the mobile phase via an intermediate flushing step with 2-propanol or ethanol the lifetime and separation performance will be affected. MACHEREY-NAGEL disclaims and excludes all warranties dealing with this kind of treatment.
RP columns: They are delivered with the eluent acetonitrile – water (40:60, v/v). For the selection of an RP eluent from acetonitrile or methanol or ethanol with pure water (filtered and degassed) can be adjusted a mixture ratio of 10:90 to 100:0 (v/v). If a buffer is used the organic part must not exceed about 50 %! For basic analytes chaotropic salts such as 1 mol/L sodium perchlorate may be used. Avoid always a pH value under 1 or about 9. Strong acidic or basic conditions can result in the dissolving of column bed or the removing of the chiral selector. The amount of buffer salts should be as low as possible. Note the solubility limit of the buffer in the eluent. Always after finishing measurements with buffer-containing eluents the column should be regenerated (see column regeneration). A changing to NP mode is not recommended. If necessary, it should only be made with an intermediate flushing step with 2-propanol or ethanol. Due to the exclusion of all warranties by this kind of treatment the use of NUCLEOCEL *DELTA* normal phases is recommended.

Flow rate and pressure
Flow rate (recommended: 0.1–1.0 mL/min) influences the time required, the resolution and the column lifetime. It is limited by the back pressure, which should not exceed the maximum of 150 bar. We recommend controlling back pressure regularly. If a high pressure results from the use of the column at nominal flow rates, this usually indicates that some contaminants have become deposited on the packing material, which must be removed (see troubleshooting).

Temperature
Column temperatures from 0–40 °C are recommended. However, they should be at least 30 °C below the boiling temperature of the eluent, in order to ensure proper detection. Variation of the temperature influences retention times and especially the peak shape. Lower temperatures benefit enantiomer separations.

Detection
UV, fluorescence, refractometric and electrochemical detectors can be used with the column. If a higher sensitivity is required, post-column derivatizations with an appropriate detector for the reaction product can be used.

Equilibration
Prior to measurement of samples the column must be rinsed with the eluent at the same flow rate and temperature as the method to be applied. Column equilibration is finished, when the baseline of the detector no longer shows a drift (generally after 10 column volumes).

Column storage
The original eluent (see eluent) at room temperature is recommended for storage. After the use of buffers and additives (e.g., amines, TFA or perchlorates) remove these to prevent corrosive effects to the stainless steel parts (see column regeneration). For column storage be sure the end fittings are tightly sealed using column end plugs, because storage without these seals can result in drying of the packing material. Under these circumstances rinse the column with approx. 10 column volumes of the eluent of storage at a flow rate of max. 0.2 mL/min.

Troubleshooting
The following outline describes the symptoms of performance loss and their cause. All columns are subject to the strict regulation and control of our quality assurance system. Columns based on silica are robust and hold their separation efficiency for long periods by correct maintenance and treatment. According to experience, column failures are mostly a result of injection of contaminants to the sorbent bed. The usage of a guard column, as well as an appropriate sample pretreatment will help to minimize these risks.
Use the outline below to help determine the cause of a possible performance loss:

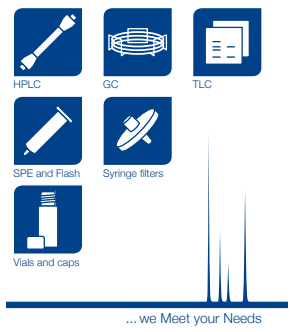
Symptom / Error / Cause	Prevention / Remedy
Baseline drift	
· insufficient period for equillibration with the eluent	longer or better equilibration
· contaminated eluent	use freshly prepared solvents and reagents
· temperature	column temperature control
Broad peaks	
· mixing and / or diffusion before / behind the column	keep length and ID of capillaries at a minimum
· too large sample volume	smaller injection volume
Peak interference; too fast elution	
too fast elution and / or insufficient separation by:	
· improper column temperature or flow rate	optimize concerned parameter
· elution power of eluent is too high	optimize eluent system
Increasing back pressure; degradation of the separation performance	
contamination of sorbent by:	
· particulate accumulation on frit or sorbent bed from sample, eluent or system	prepare fresh eluent; prefilter samples and eluent, use in-line filter / rinse LC system, clean the sorbent
· removing of chiral selector	ever use allowed eluent / replace column
Insufficient separation; degradation of the separation with regular column pressure	
contamination by:	
· coating of sorbent surface with organic substances from improperly prepared eluent or samples	remove organic substances by sample preparation / clean the sorbent (see column regeneration)
· removing of chiral selector	ever use allowed eluent / replace column
Double peaks (dead volume)	
· faulty fittings (capillaries, ferrules, nuts)	use “PEEK Fingertight Fittings”, REF 718770 / replace fittings
· dissolution of silica by too high pH value of eluent	consider pH range of column / replace column

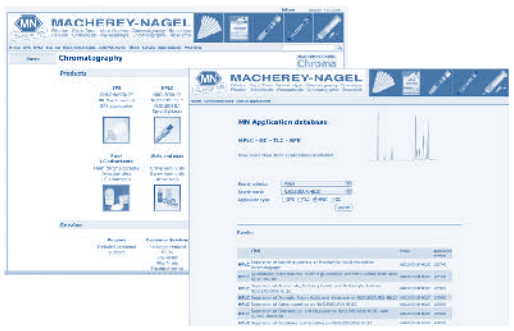
Column regeneration
In some cases the perfomance of the column can be restored by removing contaminants from the sorbent bed or by regeneration of the phase. It is important, however, to locate the source of contamination before using the column for the analysis of samples again.
1. Prepare fresh eluent: Sometimes the performance loss is caused by eluent contamination. Therefore, prepare fresh eluent and flush all liquid lines before using the column again. The eluent should be filtered through a 0.2–0.45 µm membrane and degassed prior to use.
2. Cleaning of sorbent: To remove contamination or buffer additives from RP columns rinse the column with a minimum of 10 column volumes (see table below) at the original flow rate and temperature as follows:
   NP columns:
   · 100 % ethanol to remove polar organic compounds
   · if necessary, with inverse flow direction at 1/5 of original flow rate
   · column is converted to storage condition with *n*-heptane – 2-propanol (90:10, v/v) at original flow rate
   RP columns:
   · acetonitrile – water (10:90, v/v) to remove the buffer *(also after finish of measurement series)*
   · acetonitrile – water (90:10, v/v) to remove non polar organic compounds
   · if necessary, with inverse flow direction at 1/5 of original flow rate
   · column is converted to storage condition with acetonitrile – water (40:60, v/v) at original flow rate.
   An adequate indicator for a clean column is a constant baseline. At constant temperature you should observe less than 2–3 mAU drift during a running time of 5 min with an isocratic run.
3. Column replacement: The above procedures will restore performance only in certain cases. Some organic contaminants are particularly refractory and may not respond to treatment. Also dead volume, due to column compression can generally not be repaired. Under these circumstances, column replacement is necessary. It is highly advisable to locate the cause of the problem before installing a new column.

Length [mm]	Inner diameter [mm]:	Column volume [mL]:
150	4.6	2.50
250	4.6	4.15

Abstract
To extend column lifetime, please keep in mind the following:
1. As NP eluents *n*-heptane or *n*-hexane with 2-propanol or ethanol and as RP eluents organic aqueous eluent systems (e.g., acetonitrile – water or buffer) are recommended. Eluents should be filtered through a 0.2–0.45 µm membrane and degassed.
2. Filter samples through a 0.2–0.45 µm CHROMAFIL® Xtra PET syringe filter before injection.
3. Use a guard column for contaminated samples.
4. The recommended flow rate is 0.1–1.0 mL/min.
5. Adjust flow rate to keep column pressure below 150 bar.
6. Store the NP column in *n*-heptane – 2-propanol (90:10, v/v) and the RP column in acetonitrile – water (40:60, v/v).
7. Use analytical grade reagents and HPLC grade solvents for all work. Discard any solutions that show evidence of bacterial growth.

Please check the full range of MACHEREY-NAGEL chromatography products!





… for applicative support please visit our website
with more than 3000 chromatography applications: ***www.mn-net.com/apps***

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