RESOLVOSIL BSA-7

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column.
The RESOLVOSIL BSA-7 column is a quality product based on the robust silica NUCLEOSIL®. The protein BSA covalently bonded to silica is naturally sensible for denaturation. Thus, the lifetime of the column highly depends on the measurement and the treatment of the column. 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 been specifically developed for the chromatographic separation of optical isomers and demonstrated to be a succesful tool for optical resolution as well as for 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., propanol) 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).

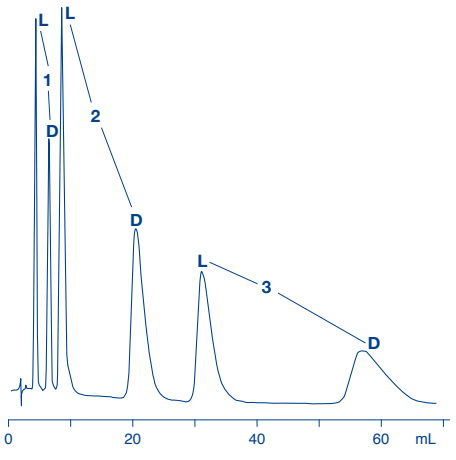
Description of the column
The stationary phase RESOLVOSIL BSA-7 is based on bovine serum albumin (BSA) covalently bonded to wide-pore spherical silica. Chiral recognition is based on the selective interaction between the protein BSA and low molecular compounds. Also hydrophobic interaction as well as interaction from polar groups and sterical effects influence the separation mechanism. The column has proven useful for chiral analysis of amino acid derivatives, aromatic amino acids, aromatic sulfoxides, barbiturates, benzodiazepinones, bezoin and benzoin derivatives, β-blockers as well as coumarin derivatives and also for monitoring stereoselective microbial and enzymatic conversions. The main advantages of the RESOLVOSIL column are extremely high selectivity and easy regulation of retention by small changes in the mobile phase composition. This results in a high flexibility of the chromatographic system because an optical resolution may be optimized to fit given requirements.

Application note

Enantiomer separation of *N*-benzoyl-*D,L*-amino acids

Column: EC 150/4 RESOLVOSIL BSA-7
Eluent: 50 mmol/L phosphate buffer, pH 6.5 + 1 % 1-propanol
Flow rate: 0.70 mL/min
Detection: UV, 225 nm

Peaks:
1. Serine
2. Alanine
3. Phenylalanine



S. Allenmark et al. in „Affinity Chromatography and Biological Recognition“ (I. Chaiken, M. Wilchek and I. Parikh, eds.), Acad. Press New York (1983), 259–260

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Further application notes can be found in our application database in the internet under ***www.mn-net.com/apps***.

Installation
The column should be installed in the flow direction indicated on the column label. It is 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. Injection of aqueous samples can be made directly onto the column.But sample solutions should be passed through a syringe filter (e.g., CHROMAFIL® Xtra PET, 0.45 µm, 25 mm, REF 729220) before entering the column. If injected sample solutions 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. A good resolution is achieved with sample concentrations below 0,2 µmol per injection.

Eluent
The RESOLVOSIL BSA-7 column is supplied with the eluent 0.1 mol/L phosphate buffer, pH 7.5 + 2 % 1-propanol. The column is compatible with mobile phase systems consisting of aqueous buffers with a pH between 5 and 8. Phosphate and borate buffers are adequate for this purpose. Avoid the use of pH extremes (< 5 or > 8). Retention and optical resolution can be regulated via pH, buffer strength (0.01–0.2 mol/L) and / or surface tension via small amounts of 1-propanol (0–5 %) added as a co-solvent. Retention is always drastically reduced by as little as 1–2 % 1-propanol and > 5 % is not recommended. pH and ionic strength will affect retention in a way not generally predictable. To some extent the columns respond like reversed phase columns, but please note that the columns will not tolerate mobile phase systems containing acetonitrile or methanol because the protein will be denatured. Eluents should be always filtered through a 0.2–0.45 µm membrane filter and degassed.

Flow rate and pressure
Flow rate (recommended: 0.5–1.5 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 300 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 10–40 °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. Optimum temperatures for successful separations should be determined empirically.

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 0.1 mol/L phosphate buffer, pH 7.5 + 2 % 1-propanol and cooling in a refrigator is recommended for storage. 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 equilibration 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
· denaturation of protein	never use acetonitrile or methanol; addition of propanol under 5 % / 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)
· denaturation of protein	never use acetonitrile or methanol; addition of propanol under 5 % / 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 traced to 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 and for regeneration of phase rinse the column with pure water at a flow rate of 0.5 mL/min for approx. 4–5 h. Then change to the original working or storage conditions. 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 or a denaturation of protein 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.

		Column volume [mL]			
Length [mm]	**Inner diameter [mm]:**	2	3	4	4.6
150		0.45	1.05	1.90	2.50
250		0.80	1.75	3.15	4.15

Abstract
To extend column lifetime, please keep in mind the following:
1. As eluents aqueous buffer systems in the pH range of 5 to 8 are recommended (e.g., phosphate or borate buffer). The addition of up to 5 % 1-propanol is possible. 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 0.5–1.5 mL/min.
5. Adjust flow rate to keep column pressure below 300 bar.
6. Store the column in 0.1 mol/L phosphate buffer, pH 7.5 + 2 % 1-propanol in a refrigerator.
7. Use analytical grade reagents and HPLC grade solvents for all work. Discard any solutions that show evidence of bacterial growth.

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