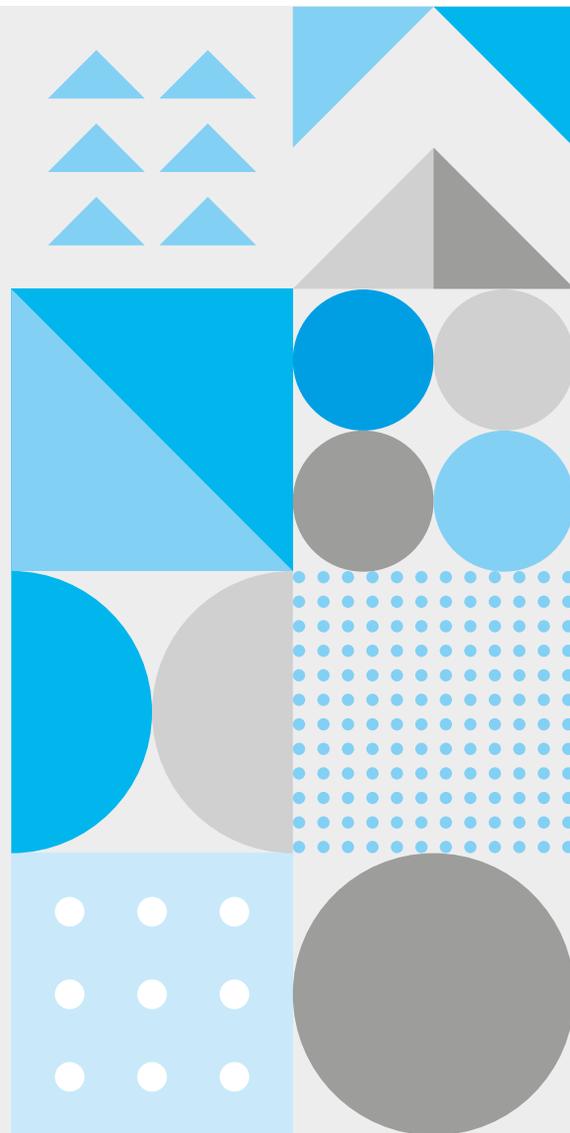


Praesto[®] NHS

Pre-Activated Resins



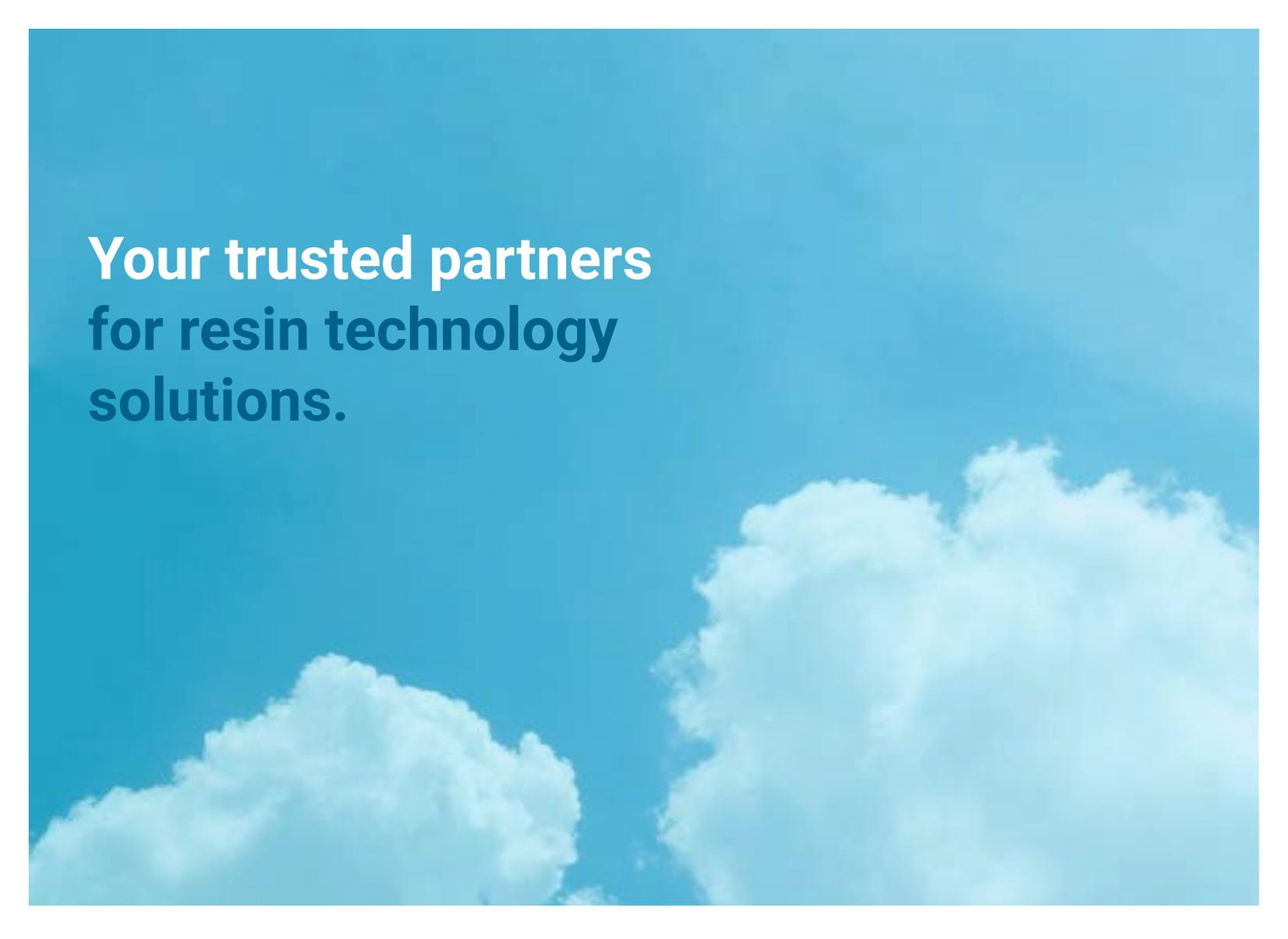


Your solutions company

Purolite Life Sciences focuses on any applications involving interactions with people, bringing Purolite's innovative thinking and distinguished history of resin technology expertise to the global Life Sciences marketplace.

We supply premium APIs, enzyme carriers, immobilized enzymes, and agarose or synthetic chromatography resins for purification and separation, to support research and development and production-scale applications in pharmaceuticals, protein purification, food processing, bioprocessing and fine chemical markets.

**“We provide solutions
for our customers’
most critical questions.”**

A background image of a clear blue sky with several large, fluffy white clouds. The clouds are positioned in the lower half of the frame, with one large cloud on the right and two smaller ones on the left.

**Your trusted partners
for resin technology
solutions.**



**Modern, high flow
agarose-based, NHS-activated
resin for immobilization of
ligands, mAbs, proteins and
other molecules.**

Praesto NHS Resins

To support in the development and manufacture of biopharmaceuticals, Purolite has developed a range of pre-activated agarose resins. These resins enable manufacturers to couple their own ligands to develop affinity chromatography solutions. NHS, Epoxy and CNBr pre-activated chemistries are available in three particle sizes - 45 μm , 65 μm and 90 μm .

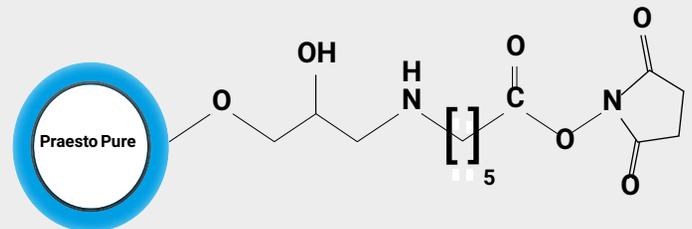
Praesto NHS resins have been designed to offer a simple solution for the immobilization of ligands onto an agarose chromatography matrix, which can be utilized to make customized affinity resins. This enables rapid scale-up from R&D proof of concept to larger scale bioprocess production columns.

Praesto NHS resins couple ligands which have a primary amine. Praesto NHS incorporates a spacer chain which separates the ligand from the chromatography matrix enabling maximum accessibility to the ligand.

The pre-activated NHS resin forms a stable amide linkage between the matrix and ligand resulting in very low ligand leakage and high caustic stability.

A common application for NHS resins is immobilization of antibodies to create immunosorbents. Coupling is via primary amino groups. Many well documented references are publicly available.

Praesto NHS Pre-Activated Resin Structure



Praesto NHS Key Performance Benefits

**Key Performance
Benefits**

**Very low levels of
non-specific binding**

Higher flow velocities

**Customizable
particle sizes**

Established chemistry

**Highly chemically
stable**

Simple ligand coupling

**Improved process
economics**



**“Purolite Life Sciences
is the only provider
in the world capable
of supplying these
enhanced, uniform
beads in process-scale
volumes.”**

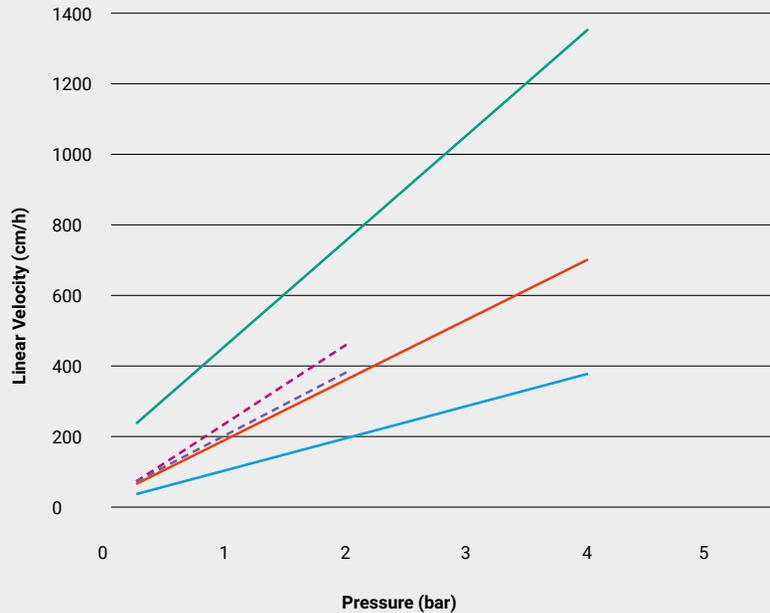
Matrix Characteristics

The Praesto NHS range of pre-activated chromatography resins use a modern, highly cross linked-agarose matrix formulation. Due to the unique rigidity and open pore structure of the Praesto agarose base beads, the Praesto NHS range is well suited for process scale chromatography to allow large columns to be operated. Proteins and other molecules containing primary amino groups are coupled directly to the pre-activated gel via a spacer. The result is a chemically stable bond and high level of biological activity between the immobilized ligand and the base matrix.

Figure 2 shows the pressure flow properties of Praesto Pure90, Praesto Pure65 and Praesto Pure45 when compared to Sepharose 4 Fast Flow (90 μm) and Sepharose 6 Fast Flow (90 μm). Even at process scale, with larger diameter columns and bed heights, the rigidity of Praesto allows processes to operate at higher flow velocities. The ability to run at high flow rates increases productivity and improves facility throughput.

Praesto NHS pre-activated resins are available in 3 particle sizes , 45 μm , 65 μm and 90 μm . Across the range of three bead sizes, porosity and ligand density is maintained. This enables the selection of an optimal particle size for a particular downstream process to maximize productivity, resolution, and pressure restraints.

Matrix Characteristics cont.



--- Sepharose 6 Fast Flow (90 µm)

— Praesto Pure90 (90 µm)

--- Sepharose 4 Fast Flow (90 µm)

— Praesto Pure65 (65 µm)

— Praesto Pure45 (45 µm)

Figure 2: The figure shows the pressure flow properties of Praesto Pure90, Praesto Pure65, Praesto Pure45, Sepharose 6 Fast Flow and Sepharose 4 Fast Flow.

Praesto Pure90, Praesto Pure 65, Praesto Pure45 were packed at 4 bar to a bed height of 20 cm in a HiScale™ 26/40 column.

Sepharose 6 Fast Flow and Sepharose 4 Fast Flow were packed at 2 bar to a bed height of 20 cm in a HiScale™ 26/40 column.

Operation and Use

Praesto NHS is supplied in 100% isopropyl alcohol and shipped under ambient conditions. It is recommended to store the resin between 2-8°C. Washing with at least 3 column volumes is required to remove the solvent prior to ligand coupling. The coupling reaction is quick and spontaneous. Shelf life is expected of at least 24 months when stored in 100% IPA and between 2-8°C.

Instruction protocols are provided in subsequent pages of this document and describe generic conditions, however we recommend specific optimization for individual processes.

The Praesto Range

The Praesto range offers a selection of modern, high-flow Affinity and Ion Exchange agarose resins, delivering exceptional results from Protein A to high-resolution polishing steps. The range also includes a full selection of Praesto Pure base matrices, and pre-activated resins in a variety of source chemistries.

All Praesto products provide an advanced, high-flow, highly cross-linked agarose base matrix. The entire range benefits from excellent pressure/flow characteristics and stability for optimal recovery of active proteins.



Placing your order



How to order

To place your order simply contact us via email or telephone using the information on the next page, and quote your order number from the table in this document.

For scale up/validation, pilot manufacturing and cGMP manufacturing operations we have an agreement with Repligen® to pack Praesto resins in 0.5 cm diameter to 80 cm diameter OPUS® columns with flexible bed heights.

If you would like to discuss how Praesto NHS can benefit your purification process, we have dedicated experts on-hand across the globe to provide knowledgeable, same-day technical assistance.

CIP, Shelf Life and Storage

Regular cleaning-in-place (CIP) is a key process step that regenerates the resin, extending lifetime and maintaining capacity through the removal of contaminants bound but not removed during a low elution pH. CIP should be optimized for each specific process, however in general the use of low and high pH solutions (e.g. 0.1 M sodium acetate containing 0.5 M NaCl, pH 4.5 and 0.1 M Tris HCl containing 0.5 M NaCl, pH 8.5) is suitable.

Ethanol concentrations using several column volume washes between 30-70% can be used to remove strongly bound contaminants.

If the coupling ligand is stable under high alkaline conditions, the use of 0.1 M NaOH is recommended. Exposure time up to 1 hour can be used but frequency, concentration and contact time should be specifically determined for the coupled ligand.

Long term storage of Praesto NHS resins should be placed in 100% IPA, below 8°C. When stored in these conditions an 18 month shelf life can be expected. Ongoing since 2014 is a long-term stability study of the Praesto base matrix. The stability of the coupled matrix is directly dependent to the coupled ligand. NHS chemistry has been successfully used for many years.

Praesto NHS Ordering Information

PRODUCT	PACK SIZE	ORDER NUMBER
Praesto NHS90	25 ml	PR01296-166
Praesto NHS90	100 ml	PR01296-164
Praesto NHS90	500 ml	PR01296-165
Praesto NHS90	1 L	PR01296-310
Praesto NHS65	25 ml	PR01292-166
Praesto NHS65	100 ml	PR01292-164
Praesto NHS65	500 ml	PR01292-165
Praesto NHS65	1 L	PR01292-310
Praesto NHS45	25 ml	PR01290-166
Praesto NHS45	100 ml	PR01290-164
Praesto NHS45	500 ml	PR01290-165
Praesto NHS45	1 L	PR01290-310

Protein Coupling to Pre-Activated Praesto NHS

Ligand Coupling Methodology

Magnetic stirrer bars should be avoided as agarose resin is susceptible to damage from grinding. Damaged agarose can result in inability to couple the ligand to the resin and poor performance of the coupled resin in the application use.

Direct heating of the solution should be avoided.

- Coupling conditions between pH 6-9 can be used, at higher pH levels the NHS ester can undergo rapid hydrolysis removing the ability to couple ligands to the resin.
- A standard buffer which can be used is 0.1 M NaHCO₃, 0.5 M NaCl (pH 8.3).
- After coupling the ligand to the resin any remaining NHS ester groups need to be deactivated from the resin, this is achieved by addition of a primary amine or thiol containing small molecules such as ethanolamine.

Coupling Procedure

Wash Praesto NHS on a filter with coupling buffer and the gel re-suspended in coupling buffer to form a slurry.

Dissolved ligand in small amount of coupling buffer and added to the suspended slurry under agitation for 4-16 hours at 20-40°C. Coupling times can vary depending on ligand concentration, pH and temperature.

The slurry composition should ideally consist of a 0.5-1:1 ratio of buffer to preactivated resin - i.e. 50 ml of gel in a total slurry volume of 75 - 100 ml. If the slurry is to dilute then reaction times are increased and incomplete coupling can occur.

Once the reaction is complete wash the coupled media on a filter with water and transfer the dewatered gel back to the reaction set up and add an equal volume of 1 M ethanolamine and stir over night at 20°C or for at least four hours at 40°C to deactivate remaining epoxide groups.

Wash the coupled resin with at least 3 equivalent volumes of acetate buffer (pH 4) followed by 3 equivalent volumes of Tris-HCl buffer (pH 8) and then 3 equivalent volumes of water. For long term storage wash the resin with 20% Ethanol solution and store the resin in 20% Ethanol solution.

Packing and Column Evaluation of Immobilized Resins

Column Packing

Packing Tricorn columns

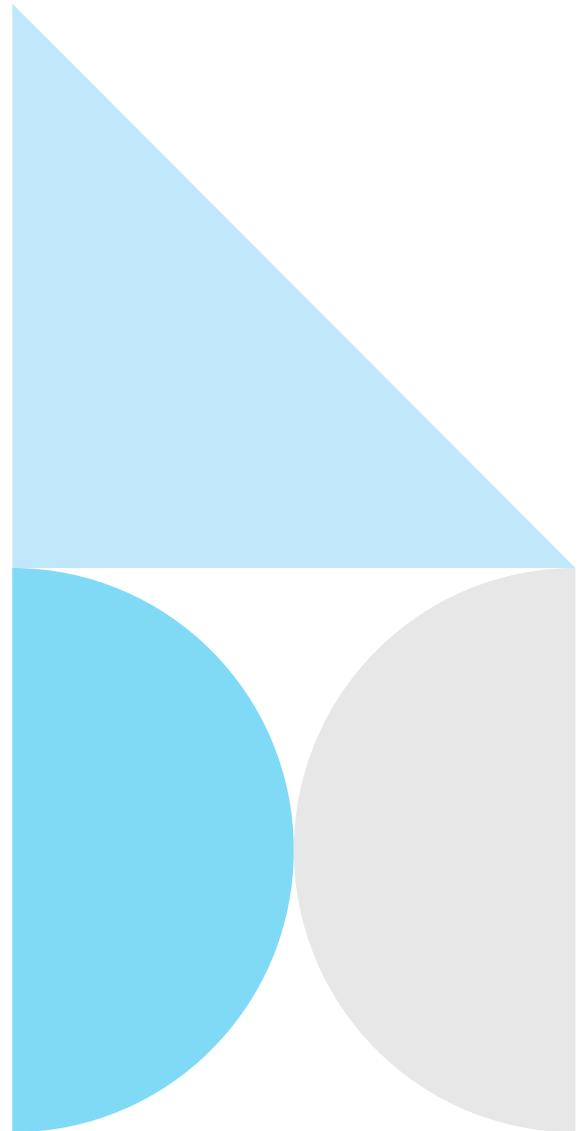
- The following instructions are for packing a Tricorn 10/300 (GE Life Sciences) column with a 30 cm bed height.
- For more details about packing Tricorn columns, please refer to the GE Life Sciences instructions: Tricorn Empty High Performance Columns (28-4094-88).

Materials and Equipment

- Praesto Pure90, Praesto Pure65 or Praesto Pure45
- Tricorn 10/100 packing equipment
- Tricorn 10/300 column
- Plastic beaker
- Plastic syringe
- Measuring cylinder
- 0.5M NaCl solution
- A Chromatography system, such as ÄKTA™ system, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.

Packing Procedure

1. Wash the sample with 5 times with 50 ml of 0.5 M NaCl solution to remove the 20% ethanol storage solution.
2. Decant off remaining NaCl wash solution and add 0.5 M NaCl solution to obtain a 70% slurry concentration.
3. Calculate the required slurry volume for a 30 cm packed bed.
 - a. Determining the slurry volume for column packing.
 - b. Determine the desired packed bed height.
 - c. Calculate the column volume (C_v) of a packed column by the following equation;
 - i. Cross-sectional area of the column (CSA) \times bed height (Bh)
 - ii. Multiply the column volume by a compression factor (C.F) ($C_v \times C.F$) (C.F = 1.12 to 1.15 dependent on particle size. $\approx 45 \mu\text{m} = 1.12$, $65 \mu\text{m} = 1.15$ and $= 90 \mu\text{m} = 1.15$)
 - iii. Divide by the slurry concentration (normally between 50% to 70%).



Packing Procedure cont...

d. Example calculation

Column: Tricorn 10/300

Desired bed height: 30 cm

Slurry concentration: 70%

Compression Factor (90 µm): 1.15

$(CSA \times Bh \times C.F.) / (\text{Slurry Concentration})$

$((0.5)^2 \pi) \times 30 \times 1.15 / 0.7 = 38.7 \text{ ml}$

Required slurry volume for a 30 cm packed bed = 38.7 ml.

4. Unpack a Tricorn 10/300 column, assemble and connect Tricorn 10/100 packing equipment as per the manufacturer's instructions (GE Life Sciences).

5. Stir column media gently with a plastic spatula (DO NOT use a magnetic stirrer bar to ensure homogeneity) and pour down a plastic spatula into the top of the packing column until the column and packing column are completely full. Leaving an Inverted meniscus at the top of the packing column.

6. Insert connector, with filter attached, at a 45° angle to prevent air bubbles forming at the top of the column and screw the top cap of the packing column.

7. Using an ÄKTA™ system, start a flow rate of 0.5 ml/min of 0.5 M NaCl packing solution through position 1 of the column valve. Once a flow is established, connect 0.5 mm tubing from column position 1A to the top of the packing column.

8. Remove the stop plug from the bottom of the column and replace with 0.5 mm tubing running into a waste container.

9. Adjust the flow rate to 1.25 ml/min and run until the resin has settled, then increase the flow rate to * ml/min and run for ** minutes to pack the resin. (Praesto Pure45 does not require a settling step, please skip to step 10)

Packing Procedure cont...

10. Stop the flow and mark the point at which the resin has settled. If necessary, remove excess medium by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula.

11. Insert column adaptor into the top of the column at a 45° angle and screw plunger down to the marked point and reconnect tubing to the top of the column from position 1A of the column valve.

12. Connect tubing from the bottom of the column to position 1B of the column valve.

13. Pack for a further 20 minutes at *** ml/min. At the end of 20 minutes mark the point at which the resin has settled.

14. Detach the tubing connected to the column and place a stop plug in the bottom of the column. Remove the lock on the top of the adaptor and screw the plunger down to the point marked in step 13.

15. Reconnect the tubing as described in step 7.

Product	Packing Flow (Pre-adaptor)*	Time**	Packing flow (Post adaptor)***
Praesto NHS90	5 ml/min	4 mins	5 ml/min
Praesto NHS65	5 ml/min	4 mins	5 ml/min
Praesto NHS45	6 ml/min	3 mins	6 ml/min

Column Efficiency Testing

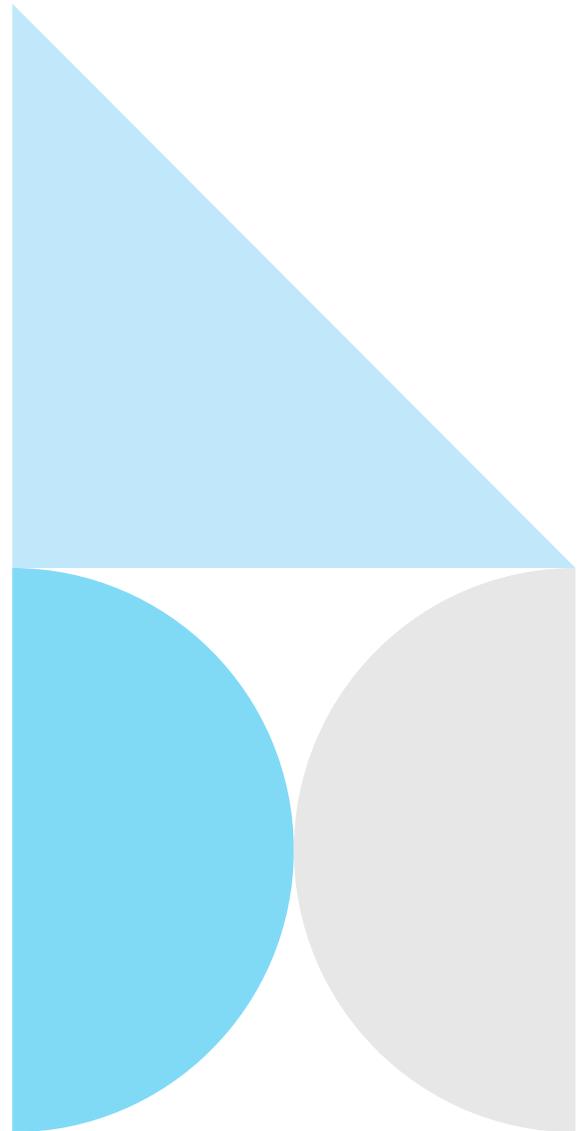
The column efficiency should be tested immediately after packing and at regular intervals during use to monitor any deterioration.

The preferred method for determining the efficiency of a packed column is through the use of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (AS).

The HETP and AS values are determined by applying a sample such as 1 – 3% acetone in demineralised water to the packed column.

A sample of 0.4 to 0.8 M NaCl in demineralized water can also be used.

A sample volume of approximately 1% of the column volume and a flow velocity of between 30 to 50 cm/h will give the optimal results.



Calculating HETP and A_s

Below is the calculation by which HETP and A_s are determined. This is done using the UV curve (or if using a NaCl sample, the conductivity curve is used).

$$HETP = \frac{L}{N}$$

L = bed height (cm)
N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h} \right)$$

V_R = volume eluted from the start of the sample application to the peak maximum.

W_h = The width of the recorded peak at half of the peak height.

V_R and W_h have the same units.

The reduced plate height is calculated by the following equation;

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = mean particle size (cm)

The reduced plate height is often taken into consideration when evaluating column packing efficiency. As a guide a value of < 4 well packed can indicate a well packed column. A value < 3 is considered a very good result.

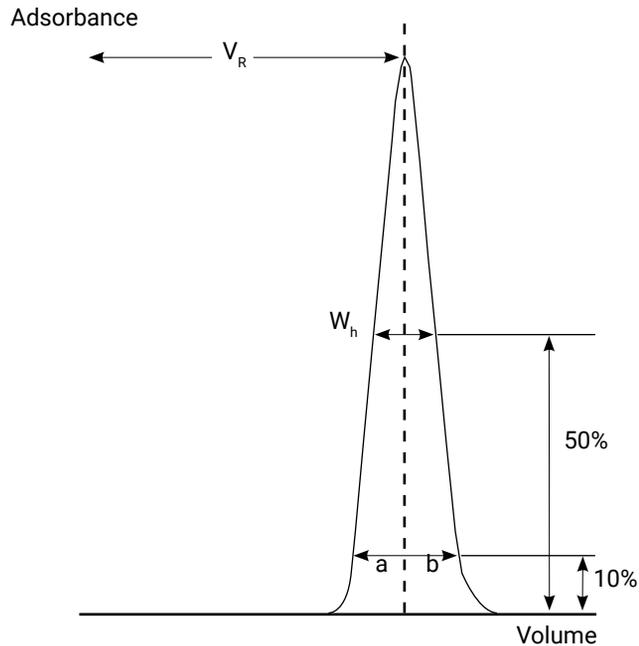
The peak corresponding to the acetone or NaCl sample should be symmetrical with an asymmetry factor as close to 1 as possible.

An acceptable is $0.8 < A_s < 2.0$

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height.
b = descending part of the peak width at 10% of peak height.

A change in the shape of the peak is usually the first indication of bed deterioration as a result of excessive use.



The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, temperature will all affect the results.

Figure 2. An example UV chromatogram of a 1 – 3% acetone sample during a column efficiency test.



Purolite Life Sciences brings Purolite's innovative thinking and distinguished history of resin technology expertise to the global Life Sciences marketplace.

Over three decades, Purolite has grown into the world's premier resin technology manufacturer and innovation leader, with production plants and advanced research labs across the globe.

Since 1981, Purolite® has grown into the world's premier resin-based separation, purification and extraction technology manufacturer and innovation leader, with manufacturing facilities, advanced research laboratories and over 1400 people employed world-wide.





100% focused on resin technology.



Global manufacturing at facilities in the UK, Romania, China and USA.



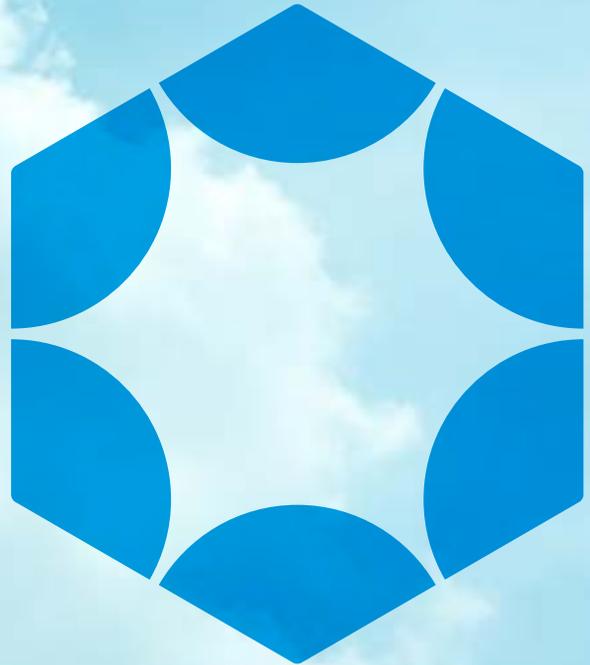
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Over 35+ years of experience in solving advanced R&D and purification challenges.



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