

Praesto[®]
PROTEIN PURIFICATION

NHS Pre-Activated Resins

Functionalized on a modern, high flow agarose base matrix for simplified ligand immobilization and customizable affinity chromatography purification solutions

 **Purolite**[®]
Life Sciences



www.purolite.com/life-sciences

Why Purolite®?

For over 35 years, Purolite has supplied specialty ion exchange resin technology to industries within complex regulatory environments, including biotechnology, pharmaceutical, food, fine chemical and electric power generation. Purolite is the only global company to focus 100% on resin technology.

Security of Supply

Ensuring reliable availability of products in case of emergency is vital to customers and of paramount importance to Purolite.

As a leading supplier of resin media to the world's most regulated industries, Purolite has a real-world security-of-supply system in place to support your process requirements for business continuity in the instance of natural disaster or emergency.

Purolite has manufacturing facilities at 3 strategic global locations in the USA, Asia and Europe, and is currently building its 4th manufacturing plant in the UK. This facility will be the second largest agarose manufacturing plant globally, with a capacity of 100,000 L per annum.

Currently, approximately 90% of all biopharmaceuticals approved by the U.S. Food and Drug Administration utilise a single source of agarose resins from a single manufacturing site, presenting a security of supply risk to long-term clinical trial material production.

Purolite have addressed this industry-wide concern by providing the first proven and reliable alternative source of agarose resins, allowing customers to dual-source their products to mitigate their supply risks.

Regulatory Support

Purolite Life Sciences provides customers with regulatory support documentation for **Praesto** products used by our customers in GMP regulatory environments.

Comprehensive regulatory support files are available for each **Praesto** resin, and are provided under a confidential disclosure agreement.

The purpose of this Regulatory Support File (RSF) is to provide assistance with:

- ◆ [Process development of clinical and commercial purification processes](#)
- ◆ [Manufacturing validation](#)
- ◆ [Quality control tests](#)
- ◆ [Standard Operating Procedure \(SOP\) for cleaning in place \(CIP\) and sanitization](#)
- ◆ [Application for various regulatory licenses or compliance](#)
- ◆ [Plant and document audits](#)

Quality

Global ISO 9001:2008 standards ensure consistent operating practices across each of our plants. Compliance is monitored and maintained through a quality assurance and regulatory team who conduct internal audits to ensure operations meet the guidelines and protocols for equipment and procedures.

Additionally, our production team is given continual training on quality processes to ensure batch-to-batch consistency, and we host numerous customer audits each year to make sure that we are in compliance with user expectations.

Purolite maintains a global Quality Management System (QMS) which supports BSI requirements of ISO 9001:2008.

Raw Materials

Our raw material suppliers are selected and qualified from leading manufacturers and are part of our global network of suppliers. Each key raw material has at least one alternative supplier and is managed through a globally coordinated inventory system to ensure security of supply.

Additionally, a quality control protocol is in place for testing new batches/lots of raw materials to confirm product specifications and lot-to-lot consistency.

Purolite Life Sciences also has long-term supply agreements in place for our Protein A ligands, which are sourced from Repligen Corporation.

Repligen provides dual-site supply for critical raw materials and has a long-standing history of successfully supplying a variety of Protein A ligands to the industry.



100% focused on resin technology.



The world's second largest agarose manufacturing facility.



De-risked long-term supply through dual-sourcing.



25+ years of regulatory experience from FDA inspected cGMP facility.



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

Pre-Activated Base Matrices

Praesto[®] NHS

Pre-activated NHS resin functionalized on a modern, high flow agarose base matrix for simplified ligand immobilization and fully customizable affinity chromatography purification resins

Overview

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

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.

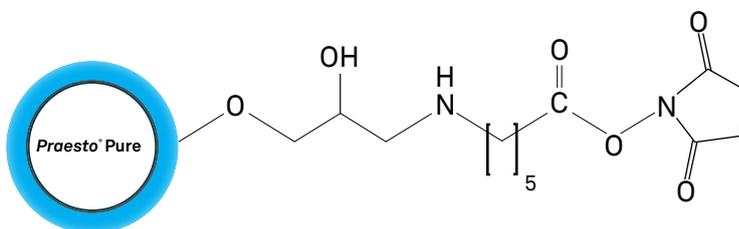
The pre-activated NHS resin forms a stable amide linkage between the matrix and ligand resulting in very low ligand leakage and high caustic stability.

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.

Key Performance Benefits

- ◆ Very low levels of non-specific binding due to the highly hydrophilic properties of the agarose base matrix
- ◆ Rigid base matrix allows significantly higher flow velocities than other pre-activated resins (e.g. Sepharose), making them suitable for process-scale operations - flow velocities are more than 100% higher
- ◆ Quick and straight forward coupling of ligands containing primary amines
- ◆ Spacer arm increases access to the NHS groups, maximizing ligand coupling and subsequent binding capacity
- ◆ Modern range of resins maximizes facility productivity, improving process economics significantly

Praesto NHS Pre-Activated Resin Structure



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 immobilised 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.

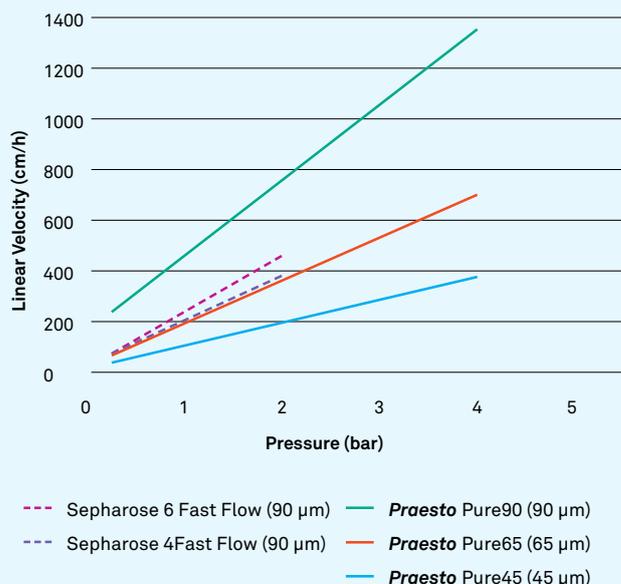
Praesto NHS pre-activated resins are available in three particle sizes, 45 µm, 65 µm and 90 µm. Across the range of three bead sizes, porosity and ligand density is maintained. This enables the selection of an optimal particle size for a particular downstream process to maximize productivity, resolution,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



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.

Ordering Information

To place your order simply contact us via email or telephone using the information on the back page of this brochure and quote your order number from the table below.

If you wish to discuss your purification challenges with a specialist, we have dedicated experts on-hand across the globe to provide knowledgeable, same-day technical assistance.

Ordering Information		
PRODUCT	PACK SIZE	ORDER NUMBER
<i>Praesto</i> NHS90	25 ml	PR01296-166
<i>Praesto</i> NHS90	100 ml	PR01296-164
<i>Praesto</i> NHS90	500 ml	PR01296-165
<i>Praesto</i> NHS90	1 L	PR01296-310
<i>Praesto</i> NHS65	25 ml	PR01292-166
<i>Praesto</i> NHS65	100 ml	PR01292-164
<i>Praesto</i> NHS65	500 ml	PR01292-165
<i>Praesto</i> NHS65	1 L	PR01292-310
<i>Praesto</i> NHS45	25 ml	PR01290-166
<i>Praesto</i> NHS45	100 ml	PR01290-164
<i>Praesto</i> NHS45	500 ml	PR01290-165
<i>Praesto</i> 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 0.2 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 molecule 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 a 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 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 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 0.5M 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) × 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%).

d. *Example calculation*

Column: Tricorn 10/300

Desired bed height: 30 cm

Slurry concentration: 70%

Compression Factor (90 μm): 1.15

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

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

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).

* ÄKTA and Tricorn are trademarks of GE Healthcare

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) 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 NHS90	5 ml/min	4 mins	5 ml/min
Praesto NHS90	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.

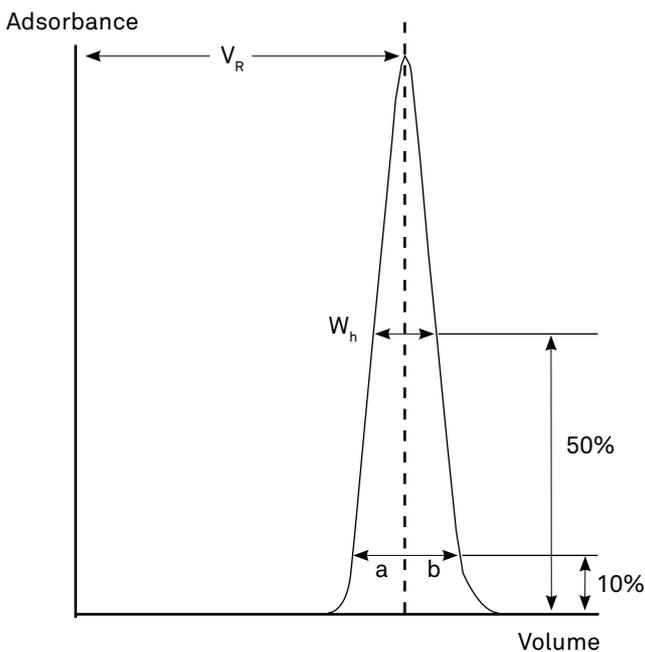
An acceptable is $0.8 < A_s < 2.0$

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

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.

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



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

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