NHS-activated **Sepharose** 4 Fast Flow

AFFINITY CHROMATOGRAPHY RESINS

The preparation and use of affinity chromatography resins by coupling group-specific ligands to preactivated resins is a widely used, successful, and well-documented technique. NHS-activated Sepharose™ 4 Fast Flow is a preactivated agarose matrix that increases the choice of coupling chemistries available (Fig 1). NHS (N-hydroxysuccinimide) coupling forms a chemically stable amide bond with ligands containing primary amino groups. NHS-activated Sepharose 4 Fast Flow provides a spacer arm and is therefore particularly suitable for immobilizing small protein and peptide ligands.

Key features of NHS-activated Sepharose 4 Fast Flow include:

- Particularly suitable for coupling of small amino-containing proteins and peptides in process-scale applications
- High level of preactivation, which gives a high degree of substitution of the selected ligand
- NHS coupling method results in chemically stable ligand attachment
- BioProcess™ resin supported for industrial applications

The advantages of high stability and a spacer arm combined with the high flow and stability characteristics of Sepharose 4 Fast Flow make this preactivated resin attractive for pharmaceutical companies producing therapeutic products. The preactivated resin can be used to prepare affinity adsorbents that can isolate specific substances from complex mixtures, often achieving very high purity in a single step.

Fig 1. NHS-activated Sepharose 4 Fast Flow has a high level of activity and gives a very stable coupled product.

Characteristics

NHS-activated Sepharose 4 Fast Flow is a cross-linked and preactivated matrix prepared by coupling Sepharose 4 Fast Flow with 6-aminohexanoic acid via a spacer arm. The terminal carboxyl group is activated by esterification with N-hydroxysuccinimide. Ligand-containing primary amino groups couple directly to this active ester to form a chemically very stable amide linkage.

To maintain high activity, NHS-activated Sepharose 4 Fast Flow is supplied as a suspension in 100% isopropanol, which should be washed away before use. Specific regulations may apply when using this product since it can require the use of explosion-proof areas and equipment. Table 1 summarizes the main characteristics of NHS-activated Sepharose 4 Fast Flow.

The high mechanical strength of the cross-linked matrix makes it well-suited for use in large columns. Scaling up a purification with a resin based on NHS-activated Sepharose 4 Fast Flow is therefore simple and predictable.

Table 1. Main characteristics of NHS-activated Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Ligand	N-hydroxysuccinimide (NHS) activated
Ligand concentration	~ 16–23 µmol NHS/mL resin
Group to be coupled	Primary amines
Particle size, d_{50v} [1]	~ 90 µm
Particle size range	45 to 165 µm
pH stability, operational[2]	2 to 9
pH stability, CIP[3]	2–13 (ligand dependent)
Pressure-flow characteristics[4]	150–250 cm/h at 0.1 MPa (1 bar, 14.5 psi) in an XK 50/60 column, 25 cm bed height (at 25°C using water)
Storage	2˚C to 8˚C, 100% isopropanol

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.
[4] The pressure-flow characteristics describe the relationship between pressure and flow under the set circumstances. The pressure given is not the maximum pressure of the resin. The pressure-flow test was performed on the base matrix.

The coupling reaction

The coupling reaction, which is rapid and spontaneous, is easy to carry out and requires no special chemicals or equipment. NHS-activated Sepharose 4 Fast Flow is supplied as a suspension. Coupling a ligand to the activated matrix involves washing the resin followed by coupling.

Instructions for use for NHS-activated Sepharose 4 Fast Flow describe methods for coupling ligands and the effect of different conditions on the coupling efficiency. Users should develop a specific procedure for each individual application.

Cleaning-in-place and sanitization

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants that may remain in the packed column after regeneration of the affinity adsorbent. Regular CIP also prevents the build-up of contaminants in the coupled NHS-activated Sepharose 4 Fast Flow and helps maintain the capacity, flow properties and general performance of the resin. A specific CIP protocol should be designed for each process according to the type of contaminants present and the stability of the coupled ligand.

The frequency of CIP depends on the nature and condition of the starting material, but one CIP cycle is generally recommended every 5 separation cycles. Sanitization inactivates microbial contaminants in the packed column and related equipment.

If ligand stability permits, a generally recommended sanitization procedure is to equilibrate the packed column with 0.1 M NaOH in 20% ethanol and allow to stand for 1 h. Only a slight decrease in the lysine content of a coupled resin was noted after 500 d of exposure to 0.1 M NaOH, *see* Figure 2. Alternatively, equilibrate with 70% ethanol and allow to stand for 12 h. This latter procedure may require working in an explosion-proof environment. Consult your local safety regulations for more information.

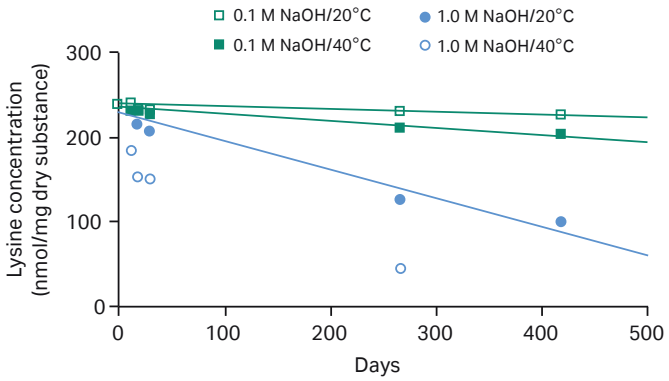

Fig 2. Residual content of lysine after storage of lysine coupled to NHS-activated Sepharose 4 Fast Flow in 0.1 and 1.0 M NaOH at 20°C and 40°C, respectively.

Resin storage

NHS-activated Sepharose 4 Fast Flow is supplied as a suspension in 100% isopropanol. When stored below 8°C, the shelf life is at least 18 mo. Note that the resin may have to be stored in an explosion-proof environment. Consult your local safety regulations for more information.

The stability of NHS-activated Sepharose 4 Fast Flow stored at 4°C to 8°C in its supplied form (100% isopropanol) was studied for up to 34 mo. The number of active NHS groups remained high after almost three years of storage. The resin will hydrolyze faster and thus lose capacity if stored incorrectly in a temperature that is too high. Note that the stability of the coupled resin is dependent on the attached ligand.

Companion product

A companion product to NHS-activated Sepharose 4 Fast Flow is CNBr-activated Sepharose 4 Fast Flow. Proteins and other molecules containing primary amino groups can be immobilized directly on this resin. CNBr-activated Sepharose 4 Fast Flow is in many cases more suited for coupling larger proteins compared with NHS-activated Sepharose 4 Fast Flow.

BioProcess resin support

NHS-activated Sepharose 4 Fast Flow belongs to the BioProcess family of products, which is developed and supported for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term resin supply, and regulatory support files (RSF) to assist process validation and submission to regulatory authorities. In addition, Fast Trak™ training and education provides high-level, hands-on training in all key aspects of process development and manufacturing.

References

1. van Sommeren A.P.G. *et al.* Comparison of three activated agaroses for use in affinity chromatography: effects on coupling performance and ligand leakage. *J. Chromatog. A* **639:** 23–31 (1993). https://doi.org/10.1016/0021-9673(93)83084-6
2. Qiujin Liang *et al.* Immobilization of Ulp1 protease on NHS-activated Sepharose: a useful tool for cleavage of the SUMO tag of recombinant proteins. *Biotechnol. Lett.* **39(7):** 1025–1031 (2017). https://dx.doi.org/10.1007%2Fs10529-017-2330-5
3. Chung, J. A. *et al.* Purification of prenylated proteins by affinity chromatography on cyclodextrin-modified agarose. *Anal. Biochem.* **386(1):** 1–8 (2009). https://doi.org/10.1016/j.ab.2008.09.007
4. Venkiteshwaran, K. *et al.* Phosphate removal and recovery using immobilized phosphate binding proteins. *Water Res. X.* **1:** 100003 (2018). https://doi.org/10.1016/j.wroa.2018.09.003

Ordering information

Product	Pack size	Product code
NHS-activated Sepharose 4 Fast Flow	25 mL	17090601
NHS-activated Sepharose 4 Fast Flow	500 mL	17090602
NHS-activated Sepharose 4 Fast Flow	5 L	17090604

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