NHS-activated Sepharose® 4 Fast Flow

Data File

Affinity chromatography

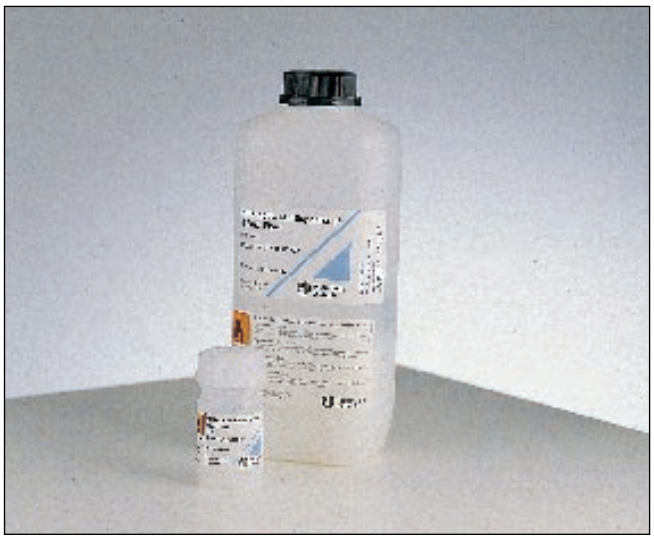
- High level of activation
- Coupled product is very stable, especially at high pH
- Spacer arm between matrix and activated group is especially suitable for immobilising small proteins and peptides
- Fast Flow matrix gives high productivity and is easy to scale up
- Comprehensive technical and regulatory support for production applications simplifies validation

Introduction

Many manufacturers of biopharmaceuticals wish to prepare separation media for affinity chromatography by coupling their own ligands to a pre-activated solid matrix. NHS-activated Sepharose® 4 Fast Flow is a recently developed pre-activated agarose matrix that increases the choice of coupling chemistries available to these users.

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 immobilising small protein and peptide ligands.

The advantages of high stability and a spacer arm combined with the high flow and stability characteristics of Sepharose 4 Fast Flow make this new coupling medium attractive for pharmaceutical companies producing therapeutic products, especially those working with synthetic peptides. The coupled gel can be used to prepare affinity adsorbents which can isolate specific substances from complex mixtures, often achieving very high purity in a single step.



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

Characteristics

Product description and use

NHS-activated Sepharose 4 Fast Flow is a bead-formed, highly cross-linked pre-activated 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. Ligands 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.

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Table 1. Characteristics of NHS-activated Sepharose 4 Fast Flow.

Sepharose 4 Fast Flow Matrix	
Mean particle size	90 µm
Particle size range	45–165 µm
Bead structure	Highly cross-linked 4% agarose, spherical
Linear flow*	150 cm/h at 100 kPa
NHS-activated Sepharose 4 Fast Flow	
Ligand density	16–23 µmol NHS/ml drained gel
pH stability**	
long term	2–13
short term (CIP)	2–13

* At 25 °C in water in an XK 50/60 column, 25 cm bed height. The flow rate after coupling may differ depending on the ligand.

** Depends on the ligand. Tested with lysine as ligand with single-point attachment.

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 matrix. Compared with NHS-activated Sepharose 4 Fast Flow, CNBr-activated Sepharose 4 Fast Flow is in many cases more suited for coupling larger proteins.

We recommend users of affinity chromatography, especially those developing purifications for scale up to production, to evaluate both products. CNBr-activated Sepharose 4 Fast Flow is described separately in Data File 18-1113-55.

Sepharose 4 Fast Flow matrix

Sepharose 4 Fast Flow is a highly cross-linked agarose matrix. In its activated form, it offers much improved performance when compared with previously available activated gels based on the Sepharose 4B matrix. The Fast Flow matrix has a higher rigidity and can thus be run at high flow rates (see Table 1). As the available capacities for proteins are similar in both cases, the Fast Flow matrix offers greater productivity.

The higher mechanical strength of the cross-linked matrix makes it well-suited for use in large columns. Scaling up a purification with a gel based on NHS-activated Sepharose 4 Fast Flow is therefore simple and more predictable. Furthermore, the medium is a member of the BioProcess® media family and carries comprehensive technical and regulatory support for production applications. The availability of this kind of information can help speed up the validation process, resulting in shortened time to market when registering a new therapeutic.

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

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 gel followed by coupling. Instructions included with the product 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 medium.

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 hr. (Only a slight decrease in the lysine content of a coupled gel was noted after 500 days exposure to 0.1 M NaOH, see Fig. 1). Alternatively, equilibrate with 70% ethanol and allow to stand for 12 hrs. This latter procedure may require working in an explosion-proof environment. Consult your local safety regulations for more information.

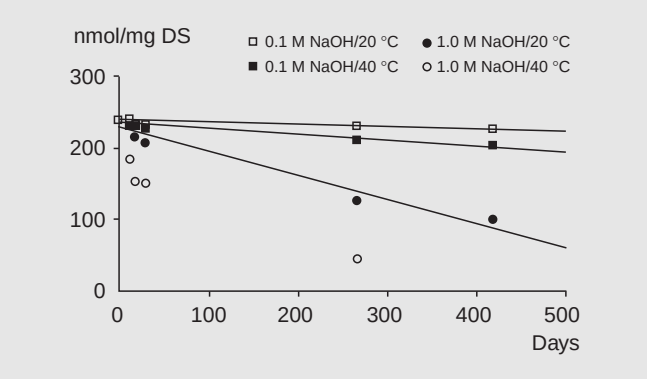

Fig. 1. Residual content of lysine after storage of ECH-Lysine Sepharose 4 Fast Flow in 0.1 M and 1.0 M NaOH at 20 °C and 40 °C.

Comparative study

A recently completed comparison of three commercially available activated gels showed that NHS-activated Sepharose 4 Fast Flow had the best performance (1). Ligand binding was very fast and complete and non-specific adsorption did not occur.

References

1. Comparison of three activated agaroses for use in affinity chromatography: effects on coupling performance and ligand leakage. *J. Chromatog. 639 (1993) 23–31*, van Sommeren, A.P.G., Machielsen, P.A.G.M., Gribnau, T.C.J.

Ordering information

Product	Size	Code No.
NHS-activated Sepharose 4 Fast Flow	25 ml	17-0906-01
	500 ml	17-0906-02

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