HiTrap™ NHS-activated HP

HiTrap is a range of prepacked columns for preparative affinity chromatography. Fast, simple, and easy separations are provided by the combination of a specially designed column and a high-performance affinity medium. HiTrap NHS-activated HP is the pre-activated member of this column family. The coupling method supplied is easy to perform; normally the affinity medium is ready to use in less than an hour. HiTrap NHS-activated HP is available in 1 ml and 5 ml prepacked columns.

Key characteristics of HiTrap NHS-activated HP columns are:

- Convenient use
- Prepacked with NHS-activated Sepharose™ High Performance
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems

The columns can easily be operated using a syringe. Alternatively, a laboratory pump (alone or within a chromatography system) can be advantageous, especially when linear gradients are required.

Media characteristics

Sepharose High Performance is the base matrix for HiTrap NHS-activated HP. The carbohydrate nature of the agarose base provides a hydrophilic and chemically favorable environment for coupling, while the highly cross-linked structure of the 34-µm spherical matrix ensures excellent chromatographic properties. Fast kinetics and high dynamic capacities are properties of all HiTrap affinity columns.

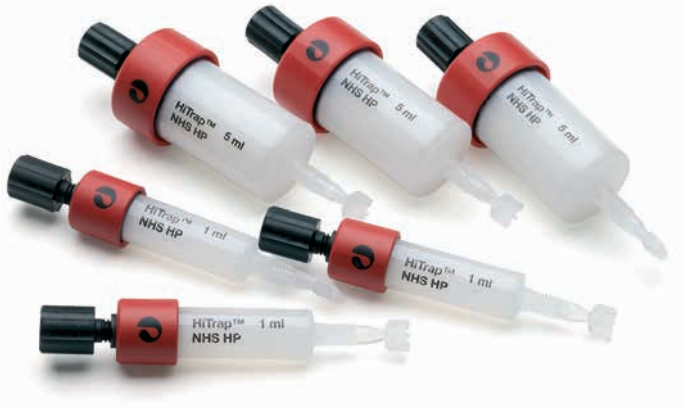

Fig 1. Prepacked with NHS-activated Sepharose High Performance, HiTrap NHS-activated HP columns allow for easy ligand coupling, allowing a wide range of uses.

HiTrap NHS-activated HP is comprised of an N-hydroxy-succinimide (NHS) ester attached by epichloro-hydrine to Sepharose High Performance via a 6-atom spacer arm. This esterification leads to the formation of activated esters, which react rapidly and efficiently with ligands containing amino groups resulting in a very stable amide linkage. The active esters are stable in the absence of water.

HiTrap NHS-activated HP is supplied in 100% isopropanol to preserve its activity prior to coupling.

Column characteristics

HiTrap columns are made of polypropylene, which is biocompatible with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet. The main characteristics of HiTrap NHS-activated HP columns are listed in Table 1.

Table 1. Characteristics of HiTrap NHS-activated HP columns

Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Ligand	NHS groups
Ligand concentration	10 µmol/ml medium
Mean particle size	34 µm
Matrix	Highly cross-linked, spherical agarose
Column hardware pressure limit	5 bar (0.5 MPa)
Maximum flow rate	4 ml/min (1 ml) 20 ml/min (5 ml)
Recommended flow rate	1 ml/min (1 ml) 5 ml/min (5 ml)
pH stability[1]	
Regular use	3–12
Cleaning	3–12
Temperature stability	
Regular use	4°C to room temperature
Storage	4°C to 8°C
Storage buffer	supplied in 100% isopropanol

[1] The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability regular use refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability cleaning refers to the pH interval for regeneration, cleaning-in-place, and sanitization procedures.
Both intervals refer to the coupled product provided that the ligand can withstand the pH.

Figure 2 shows that over 30 mg of IgG can be coupled to a HiTrap NHS-activated HP 1 ml column. The procedure takes less than 15 minutes. Further studies have shown that ligands can be coupled in the presence of detergents such as 0.1% SDS, Triton™ X-100, Tween™ 20, and sodium deoxycholate, and that the yield is independent of pH in the range of 7–10.

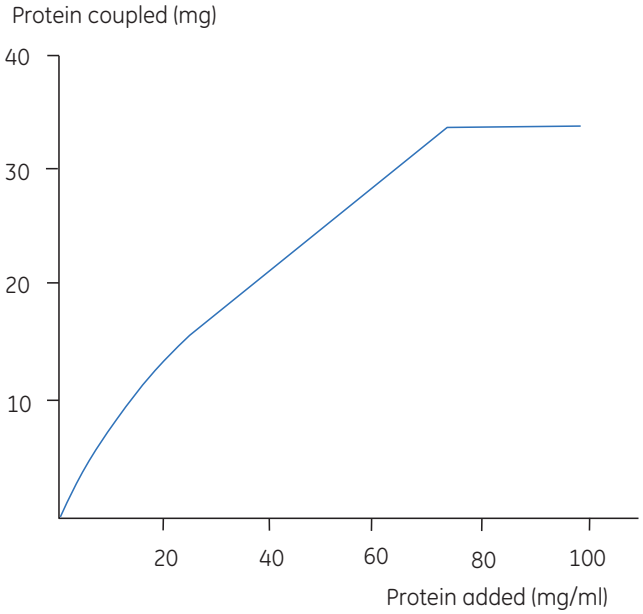

Fig 2. Coupling 30 mg of IgG to HiTrap NHS-activated HP 1 ml.

Coupling procedure

Ligands containing primary amino groups are easy to immobilize on NHS-activated Sepharose High Performance, allowing a wide range of uses that require the pinpoint specificity of a laboratory coupled affinity medium. A basic coupling procedure is described in Table 2.

Table 2. A basic coupling procedure using a syringe with HiTrap NHS-activated HP columns

Step	Notes
1. Dissolve the desired ligand in the coupling buffer to the desired concentration.	The concentration depends on the ligand being used. As a general rule: 5–10 mg/ml of ligands containing primary amino groups; the optimal volume is one column volume.
2. Remove the column's top cap.	Apply a drop of 1 mM HCl to the top of the column to avoid air bubbles.
3. Connect the HiTrap Luer adapter (or tubing from a system) to the column. Remove the column's snap-off end. Wash out the isopropanol gently.	Use 6 column volumes of 1 mM HCl at approximately 1 ml/min or 1 drop/3 sec.
4. Immediately inject one column volume of the ligand solution onto the column.	Do not allow the column to stand at this point or its coupling activity will be lost.
5. Close the column and allow coupling to take place.	15–30 minutes at 25°C (or 4 hours at 4°C). If a large volume of ligand solution is used, connect a second syringe to the outlet of the column and gently pump the solution back and forth for 10–15 minutes. A peristaltic pump, for example Pump P-1, can also be used.
6. Deactivate any excess active groups.	Inject 6 column volumes of high pH buffer (0.5 M ethanolamine, 0.5 M NaCl, pH 8.3), followed by 6 column volumes of low pH buffer (0.1 M acetate, 0.5 M NaCl, pH 4). Repeat the washing with the high pH buffer and allow the column to stand for 15–30 minutes.
7. Wash out uncoupled ligands.	Complete the washing cycle by repeating low and high pH washes as described in step 6 above, followed by an appropriate storage buffer.
8. The column is now ready for use.	Store at 4°C to 8°C.

Operation

Separations on HiTrap Blue HP columns are easily performed using a syringe and the provided Luer adapter (Fig 3), a laboratory pump, an ÄKTA system, or other chromatography systems. Instructions and connectors are included with each pack of columns.

For quick scale-up, two or more columns can be connected in series by screwing the end of one column into the top of the next. Note, however, that connecting columns in series will lead to increased backpressure.

A)

B)

C)

Fig 3. Using HiTrap NHS-activated HP with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and snap off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Elute and continue collecting fractions.

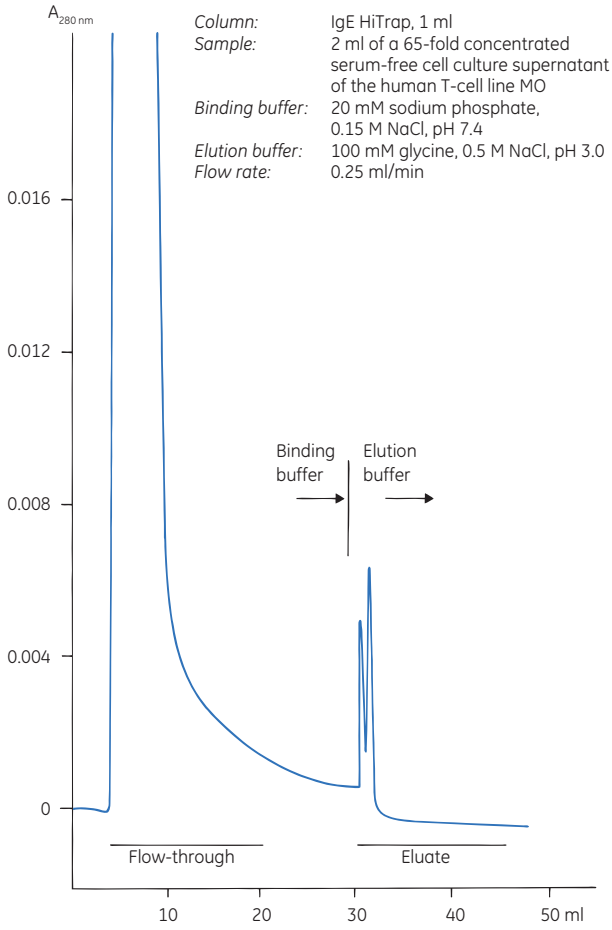

Fig 4. Separation of an IgE-stimulating factor from a human T-cell line on a 1 ml HiTrap NHS-activated HP column coupled with IgE.

Applications

The following applications show work done on HiTrap NHS-activated HP columns coupled with different ligands using the following methods:

- Single application of coupling solution by a syringe
- Circulation with two syringes
- Recirculation with a peristaltic pump

The methods resulted in excellent yields and good separations.

Partial purification of an IgE-stimulating factor from a human T-cell line

A factor that stimulates the production of IgE (but probably not one of the known interleukins) was found in an IgE-producing myeloma. Initial purification of the factor was attempted on IgE coupled to HiTrap NHS-activated HP 1 ml.

One milliliter (5 mg) of IgE was coupled to HiTrap NHS-activated HP 1 ml with a syringe. After 1 hour, the column was washed and analysis revealed that 70% (3.5 mg) of the IgE was coupled to the matrix. Figure 4 shows the subsequent separation conditions and result.

Increased production of IgE in the cell line U-266 was only found when the cells were grown in the presence of desorbed material. ß2µ levels, a reflection of total protein synthesis, showed that the increased IgE production was not due to an overall stimulus of the cell line.

Removal of BSA from an HIV-2 virus lysate

Purification of *gag* proteins from an HIV-2 virus lysate was hampered by the binding of BSA (which was present in the virus culture) to the RPC column. This BSA could not be removed completely from the column by regeneration and interfered with the HPLC purification of the viral proteins. Anti-BSA antibodies were therefore purified and coupled to HiTrap NHS-activated HP in an attempt to remove BSA from the lysate before running on the RPC column.

Coupling was carried out according to a method supplied with the column. Coupling solution was recirculated for 20 minutes at a flow rate of about 2 ml/min by manually pumping it back and forth through the column with two syringes. This method achieved 95% (81 mg) coupling of the anti-BSA.

To test the efficiency of the BSA removal, 14.1 mg of BSA were passed through the anti-BSA HiTrap column – 0.45 mg (3.2%) was detected in the flowthrough and 13.54 mg (96.0%) had bound. Total recovery of bound and nonbound BSA was 99.2%. As BSA comprised at the most one quarter of the total protein as judged by electrophoresis, more than 99% of the BSA applied to the column had probably bound.

When the HIV-2 virus lysate that passed through the anti-BSA HiTrap 1 ml column was run on the RPC column, the separation was completely free from interference by BSA.

Purification of anti-mouse Fc IgG from sheep anti-serum

Mouse IgG (10 mg, 3 mg/ml) was coupled to HiTrap NHS-activated HP at room temperature for 50 minutes by recirculation with a peristaltic pump. Analysis showed that 9.5 mg (95%) was coupled. The column was then used for purification of anti-mouse Fc IgG from sheep antiserum with excellent results.

Ordering information

Products	Quantity	Code number
HiTrap NHS-activated HP	5 × 1 ml	17-0716-01
HiTrap NHS-activated HP	1 × 5 ml	17-0717-01

Accessories	Quantity	Code number
1/16" male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16" female/M6 male	6	18-1112-57
Union M6 female/1/16" male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep™, 1/16" male connector for ÄKTA	8	28-4010-81
Stop plug female, 1/16"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector included in each HiTrap package

† Two, five, or seven stop plugs female included in HiTrap packages depending on products.

‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code number
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook, Principle and Methods	18-1022-29
Affinity Chromatography Columns and Media Selection Guide	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

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