52-2083-00 AH **Affinity media**

IgG Sepharose 6 Fast Flow

IgG Sepharose™ 6 Fast Flow, from GE Healthcare, is based on the rigid Sepharose 6 Fast Flow matrix, with human IgG covalently coupled to it. The improved mechanical characteristics of this fast flow medium allows high flow rates to be used for rapid and convenient single step purification of protein A fusion protein conjungates produced in prokaryotic expression systems. Characteristics of IgG Sepharose 6 Fast Flow are listed in table 1.

Table 1. Characteristics of IgG Sepharose 6 Fast Flow.

Ligand:	human IgG
Binding capacity:	> 2 mg protein A/ml medium at pH 7.5
Mean particle size:	90 µm
Bead structure:	highly cross-linked 6% agarose
Maximum flowrate*:	300 cm/h (98 ml/min), using XK 50/30 column with 15 cm bed height, run at room temperature with aquous buffer
Recommended flow rates*:	Sample application: < 150 cm/h (49 ml/min using XK 50/30 column)
Storage temperature:	4 to 8°C
Storage buffer:	50 mM K_2PO4, 1mM $MgCl_2$ pH 7.2 in 20% ethanol or 50mM Tris buffer, 150 mM NaCl, 0.05% Tween™ 20, pH 7.6 (TST) in 20% ethanol

* H_2O at room temperature

Sepharose

Protocol

Buffers

- Tris-saline Tween 20 (TST): 50 mM Tris buffer, pH 7.6, 150 mM NaCl and 0.05% Tween 20.
- 0.5 M CH_3COOH (HAc) adjusted to pH 3.4 with CH_3COONH_4 (NH_4Ac).
- 5 mM NH_4Ac, pH 5.0.

Procedure

WARNING! When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

Column Packing and Washing

- IgG Sepharose 6 Fast Flow is supplied as a suspension in 50mM K_2PO4, pH 7.2, 1mM $MgCl_2$, 20% ethanol as preservative.
- Pack the IgG Sepharose 6 Fast Flow suspension in any GE Healthcare column of suitable size. The column may be operated by gravity feed.
- Wash the medium with at least 5 bed volumes TST prior to use in order to remove any traces of ethanol.

Equilibration and Sample Application

- Equilibrate the column with 2–3 bed volumes each of 1) 0.5 M HAc, pH 3.4, 2) TST, 3) 0.5 M HAc, pH 3.4 and 4) TST.
- Check pH of the eluate with pH paper (should by neutral) and adjust pH of the sample, cell supernatant or clarified growth medium, if necessary. Apply the sample to the column.

Washing and Elution

- Wash the medium with
  1) 10 bed volumes TST and
  2) 2 bed volumes 5mM NH_4Ac, pH 5.0.
- Elute the sample with 0.5 M HAc, pH 3.4.
- Collect aliquots in polypropylene microcentrifuge tubes (A_{280}=1.0 for 2.6 mg protein/ml).
- The samples can be lyophilized directly without prior dialysis.
- Analyze samples by gradient sodium dodecyl sulphate polycrylamide gel electrophoresis (SDS-PAGE).

Note: This method gives a concentrated eluate and can only be used if the fusion product is stable under these conditions. For small scale purifications, gravity flow suffices throughout the procedure.

Alternative Elution Buffers

- 0.1 M glycine-HCl, pH 3.0
- 0.5 M lithium diidosalicylate dissolved in water (enzymatic activity is better preserved but some of the sample will bind to the column irreversibly). Other chaotropic salts may also be used.

Re-equilibration and Storage

- Re-equilibrate IgG Sepharose 6 Fast Flow with TST until pH of the effluent is around 7.0. This is important since IgG might denature if the chromatography medium is left standing at a low pH.
- If the medium is not going to be used for a longer period of time; wash the it matrix with 5 bed volumes 20% ethanol in TST and store at 4º to 8°C. IgG Separose 6 Fast Flow must not be frozen.

Shelf Life – General Guidelines

- Human IgG is covalently coupled to the Sepharose matrix via cyanogen bromide activation. Minute amounts of IgG that leak from the medium will be washed away in the initial washing step. No IgG is visible on a silver stained PhastGel™ Gradient 10–15 run on PhastSystem™. The sensitivity limit of this silver staining technique is 0.5 ng protein per band for PhastGel SDS-PAGE.
- Careful sample preparation will prolong the life of IgG Sepharose 6 Fast Flow. Avoid reducing agents since the disulphide bonds in IgG will be affected.
- Coloured samples may leave traces of pigment on the medium. This colouration does not influence the performance of IgG Separose 6 Fast Flow.
- The column may be operated from 4°C to room temperature, depending on the sensitivity of the protein conjugate.

Ordering Information

Product	Quantity	Code No.
IgG Sepharose 6 Fast Flow	10 ml	17-0969-01

Literature	Quantity	Code No.
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media Selectron guide	1	18-1121-86

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