CREATIVE BIOMART®
Chromatography

KappaSelect

Product Information

Cat#No#	Ka-327C

Product Overview

Antibody fragments, especially Fabs, are getting increased attention as potential biopharmaceuticals because they have some advantages over monoclonal antibodies (MAbs), KappaSelect is an affinity resin designed for the purification of human Fab (kappa) fragments.

Description

Antibody fragments are gaining increased attention as potential biopharmaceuticals because they display certain advantages over monoclonal antibodies (MAbs).
KappaSelect is affinity chromatography resins for purifying kappa and lambda Fab fragments, respectively. These resins enable efficient capture with high purity and yield.

Optimum pH

12

Characteristic

Efficient, industrial-scale capture of Fabs by affinity chromatography.
High binding capacity for Fabs.
Rigid agarose base matrix allows high flow rates and processing of large sample volumes for increased throughput.
Non-mammalian derived product reduces regulatory concerns in the production of Fabs for clinical applications.
Low ligand leakage ensures increased Fab purity and productivity.

Applications

KappaSelect is an affinity chromatography resin designed for the purification of Fab (kappa) with high binding capacity, purity and yield.

Maximum operating pressure

300 kPa at 600 cm/h

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Ligand Coupling Method
Amide linkage
Packing Column
Pack the column with resin.
Column
0.4 mL LambdaFabSelect packed in a Tricorn 5/20 column.
Matrix
Highly cross-linked agarose
Average particle size
~75 µm
Ligand
Recombinant protein (Mr 13 000), produced in S. cerevisiae, with affinity for the constant domain of the immunoglobulin kappa light chain.
Ligand density
Approx. 5 mg/ml of medium.
Dynamic binding capacity
Approximately 15 mg Fab/mL of resin
Recommended flow rate
At least 600 cm/h in a 1 m diameter column, with 20 cm bed height at 20°C using buffers with the same viscosity as water at < 0.3 MPa (3 bar).
Chemical stability
Stable to commonly used aqueous buffers: 6 M urea, 6 M guanidine hydrochloride, 70 % ethanol, 30 % isopropanol.
Physical stability

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The ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low leakage.

pH working range
3–10
pH CIP range
2–12
CIP stability
KappaSelect showed good stability up to pH 12. Use of a low pH solution or agents like guanidine hydrochloride in a cleaning protocol is therefore recommended for KappaSelect. For KappaSelect, avoid pH > 12 due to limited ligand stability under strongly alkaline conditions.
Temperature stability
4°C to 30°C
Storage
2 to 8°C, 20% Ethanol
Binding buffer
PBS, pH 7.4
Elution buffer
0.1 M glycine buffer, pH 2.5–3.0.
Equilibration
Phosphate buffered saline (PBS), pH 7.4 (0.01 M phosphate buffer, 0.0027 M KCl, 0.14 M NaCl).
Elution
Elute with 5 to 10 CV of elution buffer. Immediately adjust eluted fractions to physiologic pH by adding neutralization buffer (e.g. 1 M Tris, pH 7.5–8.5).
Cleaning-in-place
The recommended protocol comprises initial strip of the resin at low pH, and then subjecting the resin to

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NaOH of low concentration for cleaning.

Sanitization

PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2 % v/v benzyl alcohol) is used for final sanitization of the resin. PAB solution is sensitive to light and should be freshly made not to damage the resin. PAB solution should be stored in a dark bottle and kept no longer than for a week. PAB solution has a pH of < 2, and resin stability can be limited in prolonged exposure at such a low pH.

Purification procedures

A dAb expressed in the periplasm of E.coli and released by heat treatment of the bacterial suspension was used in the development of a dAb purification process. Clarification of the bacterial suspension was performed in a microfiltration step using hollow fiber filters. Capto L resin was selected for initial capture for reduction of HCP and endotoxin levels. Capto MMC ImpRes, run in bind-elute mode, was selected for the intermediate purification step for its ability to efficiently reduce HCP further. Capto MMC ImpRes is a weak CIEX multimodal resin with high selectivity in a broad pH/ salt window, allowing the use of the resin under a variety of process conditions to solve challenging purification tasks. For final polishing, Capto adhere ImpRes multimodal AIEX resin was used in flow-through mode. Like Capto Q, Capto adhere ImpRes is a suitable choice when purifying proteins with a high isoelectric point. Here, working at a pH of 8.5 allowed the used dAb to pass in the flowthrough, while impurities remained bound to the resin.

Pack size

25 mL

Wash buffer

Phosphate buffered saline (PBS), pH 7.4 (0.01 M phosphate buffer, 0.0027 M KCl, 0.14 M NaCl).

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