KappaSelect LambdaFabSelect

AFFINITY CHROMATOGRAPHY

Antibody fragments are gaining increased attention as potential biopharmaceuticals because they display certain advantages over monoclonal antibodies (MAbs). For example, Fabs show improved pharmacokinetics for tissue penetration and can bind to targets inaccessible to conventional antigen binding sites.

KappaSelect and LambdaFabSelect are affinity chromatography resins for purifying kappa and lambda Fab fragments, respectively. These resins enable efficient capture with high purity and yield. Both are part of Cytiva's Custom Designed Media program.

Benefits of KappaSelect and LambdaFabSelect include:

- 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

Resin characteristics

KappaSelect and LambdaFabSelect are based on a highly rigid agarose base matrix that allows high flow rates and low back pressure at large scale. They feature a ligand that binds to the constant region of the kappa or the lambda light chain (i.e., fragments lacking the constant region of the light chain will not bind) (Fig 1). Both resins are therefore capable of binding other target molecules that contain the constant region of the light chain, for example, IgG, IgA, and IgM. The ligands are attached to the matrix via a long hydrophilic spacer arm to make it easily available for binding to the target molecule (Fig 2). They are based on single-chain antibody fragments that are screened for either human Ig kappa or lambda.

The ligands are produced in a yeast expression system, where fermentation and subsequent purification/formulation is performed in the absence of mammalian components. Resin characteristics are summarized in Table 1.

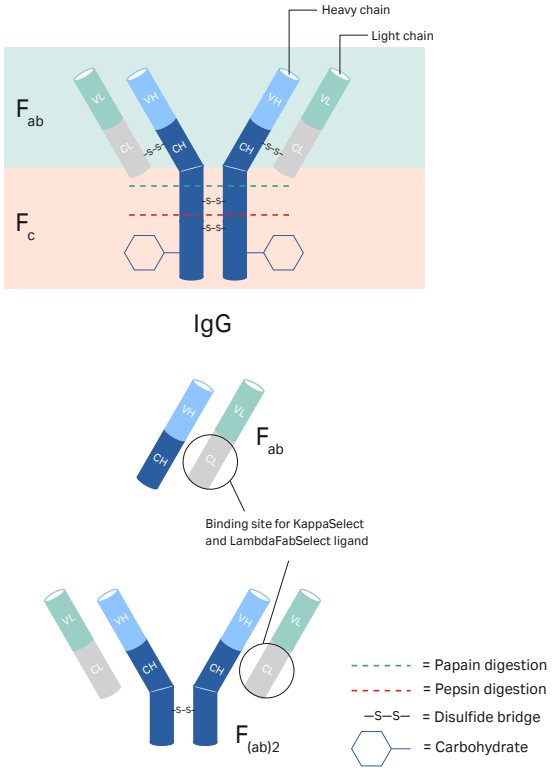


Fig 1. Antibody structure and Fab fragment binding site for KappaSelect and LambdaFabSelect ligand.

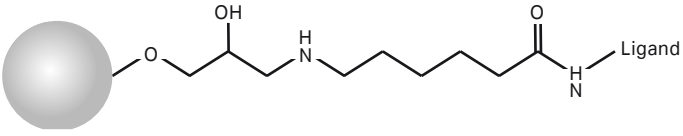


Fig 2. Partial structure of KappaSelect and LambdaFabSelect.

Table 1. Main characteristics of KappaSelect and LambdaFabSelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50V}*	75 µm
Ligand	Recombinant protein (M_r 13 000), produced in *Saccharomyces cerevisiae*, that binds to the constant region of Fab kappa or lambda light chain
Ligand concentration	Approx. 5 mg/mL resin (KappaSelect) Approx. 7 mg/mL resin (LambdaFabSelect)
Total binding capacity	Approx. 15 mg Fab/mL resin (KappaSelect)† Approx. 20 mg Fab/mL resin (LambdaFabSelect)‡
Flow velocity	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)
pH stability, operational§	3 to 10
pH stability, CIP¶	2 to 12
Working temperature**	4°C to 30°C

* d_{50v} is the mean particle size of the cumulative volume distribution.
† Protein in excess is loaded in phosphate buffered saline (PBS), pH 7.4 on a 1 mL HiTrap™ column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 0.1 M glycine at pH 2.5.
‡ Dynamic binding capacity at 10% breakthrough measured in a Tricorn™ 5/50 column, 5 cm bed height, 4 min residence time (75 cm/h) for polyclonal Fab lambda reagent in PBS, pH 7.4.
§ pH interval where the resin can be operated without significant change in function.
¶ pH interval where the resin can be subjected to cleaning in place (CIP) or sanitization in place without significant change in function.
** Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol.

Principles

Affinity chromatography is one of the chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures. The technique offers high selectivity and usually high capacity for the target molecule. As affinity chromatography is a binding technique, the sample volume does not affect the separation. Diluted samples can be applied, although capacity is commonly somewhat lower with more diluted sample.

The immobilized ligand adsorbs the target molecule under suitable binding conditions. Under suitable elution conditions, the target molecule is desorbed. These conditions depend on the target molecule, feed composition, and the chromatography resin, and they must be evaluated together with other chromatographic parameters (e.g., sample load, flow velocity, bed height, regeneration, cleaning-in-place, etc.) to establish the conditions that will bind the largest amount of target molecule in the shortest time and with the highest product recovery.

Regeneration should restore the original function of the resin. Depending on the nature of the sample, regeneration is normally performed after each cycle, followed by re-equilibration in start buffer. In order to prevent build-up of contaminants over time, more rigorous protocols may need to be applied (see *Cleaning-in-place (CIP) and sanitization* in place [SIP]).

Application

Binding and elution conditions

A typical protocol for using KappaSelect or LambdaFabSelect is described below:

Equilibration, loading, and wash buffer:	Phosphate buffered saline (PBS), pH 7.4 (0.01 M phosphate buffer, 0.0027 M KCl, 0.14 M NaCl)
Elution buffer:	0.1 M glycine buffer, pH 2.5–3.0 (KappaSelect) 0.1 M acetate buffer, pH 3.5 (LambdaFabSelect)

1. Pack the column with resin.
2. Equilibrate with 10 column volumes (CV) of equilibration buffer.
3. Load the sample.
4. Wash with washing buffer.
5. 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).

Application example

LambdaFabSelect was used to capture an antibody fragment containing the lambda light chain from an *E. coli* lysate. Figure 3 shows the chromatogram.

Yield and purity were both high. Measuring the absorbance of the elution off-line at 280 nm gave a recovery of 99%. The protein contents of the starting lysate and the various fractions were analyzed by SDS gel electrophoresis and visualized by Deep Purple staining (Fig 4). All samples were reduced with mercaptoethanol unless otherwise stated. LambdaFabSelect bound the human lambda Fab from the *E. coli* lysate, and eluted it in the elution peak.

Column:	0.4 mL LambdaFabSelect packed in a Tricorn 5/20 column
Sample:	6 mL homogenated and clarified *E. coli* lysate spiked with 1.1 mg/mL human Fab lambda
Loading flow rate:	0.1 mL/min (4 min residence time)
Binding buffer:	PBS, pH 7.4
Elution buffer:	100 mM acetate, pH 3.2
CIP:	120 mM phosphoric acid, 167 mM acetic acid, pH 1.5
Flow rate:	0.4 mL/min

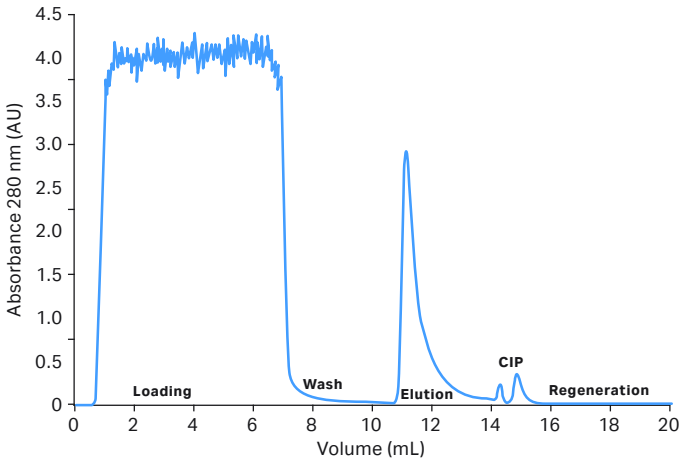
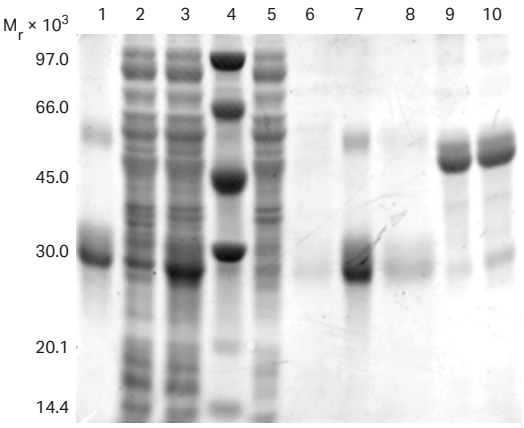


Fig 3. UV_{280} absorbance curve for loading an *E. coli* lysate and eluting human Fab lambda on LambdaFabSelect as the first capture step for lambda Fab purification.



Lanes

1. Human Fab lambda
2. *E. coli* lysate
3. *E. coli* lysate spiked with human Fab lambda (sample)
4. Low molecular weight marker
5. Flow-through
6. Wash
7. Eluate
8. CIP
9. Human Fab lambda (non-reduced)
10. Eluate fraction (non-reduced)

Fig 4. SDS-PAGE analysis (Deep Purple staining, reducing conditions) of fractions from the purification of human Fab lambda with LambdaFabSelect shown in Figure 3.

Stability

The ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low leakage. Figure 5 shows the stability of KappaSelect after storage in different solutions of various pH at 40°C during one week. Ligand leakage is low in the pH range 2 to 12, and there was only a minor effect on Fab-binding capacity when KappaSelect was stored in solutions of pH 1, 2, and 12 (one week at 20°C) (Fig 6). At pH values above 12, both carbon and nitrogen are released, which indicates hydrolysis of the ligand. An identical study has been performed for LambdaFabSelect with similar results.

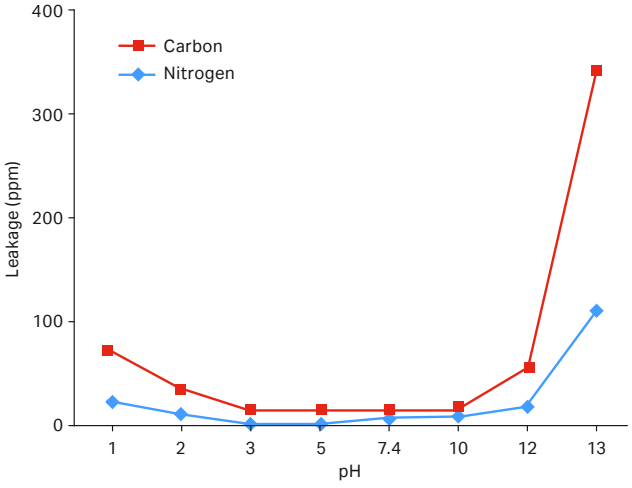


Fig 5. Stability of KappaSelect at different pH.

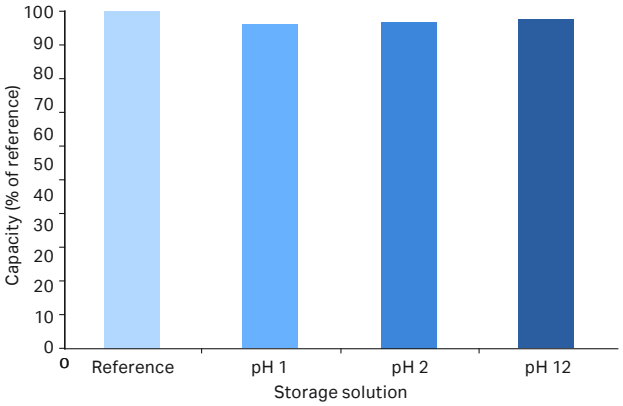
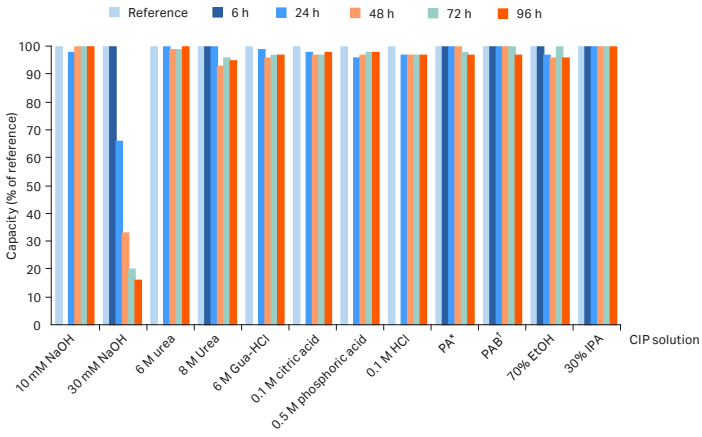


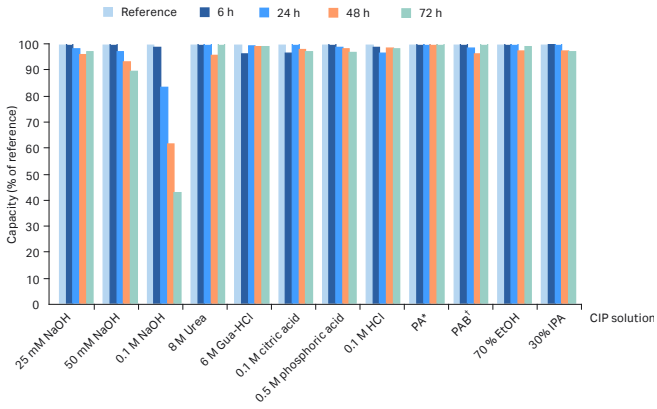
Fig 6. Fab-binding capacity (determined as percent of reference) of KappaSelect after storage in solutions of different pH.

Studies have been performed where KappaSelect and LambdaFabSelect were treated with various commonly used CIP and sanitization solutions. The Fab binding capacity was determined after set time intervals (Fig 7 and 8). KappaSelect showed good stability up to pH 12 and LambdaFabSelect at up to pH 12.7. Use of a low pH solution or agents like guanidine hydrochloride in a cleaning protocol is therefore recommended for KappaSelect. For LambdaFabSelect, mild alkali CIP solutions can be used in addition to low pH solutions. However, prolonged exposure (i.e., several days) to pH < 2 should be avoided due to slow decomposition of the agarose matrix at low pH. For KappaSelect, avoid pH > 12 and for LambdaFabSelect avoid pH > 12.7 due to limited ligand stability under strongly alkaline conditions.



* 120 mM phosphoric acid and 167 mM acetic acid
† PA and 2.2 % benzyl alcohol

Fig 7. Fab-binding capacity (determined as percent of reference) of KappaSelect after treatment with various CIP and sanitization solutions.



* 120 mM phosphoric acid and 167 mM acetic acid
† PA & 2.2 % benzyl alcohol

Fig 8. Fab-binding capacity (determined as percent of reference) of LambdaFabSelect after treatment with various CIP and sanitization solutions.

Leakage assay

For determination of ligand leakage from both products, the Thermo Scientific™ CaptureSelect™ Leakage ELISA Kit (Thermo Fisher Scientific) for the respective product can be used.

Cleaning-in-place (CIP) and sanitization

A cleaning or sanitization protocol should be designed for each application, as the efficiency of the protocol is strongly related to the feedstock and other related operating conditions. The recommended protocol comprises initial strip of the resin at low pH, and then subjecting the resin to NaOH of low concentration for cleaning. Lastly, 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.

1. 0.1 M citric acid, pH 2.1; 10 min; 13 CV
   10 CV PBS, pH 7.4
2. 10 mM NaOH, pH 12; 15 min; 19 CV
   10 CV PBS, pH 7.4
3. PAB; 15 min; 19 CV

Equilibrate the resin using equilibration buffer prior to next purification cycle. The binding capacity was tested after 25 cycles and was found to be intact.

Storage

We recommend storing the resins in 20% ethanol at 2°C to 8°C. KappaSelect and LambdaFabSelect are supplied as suspensions in 20% ethanol.

Ordering information

Product	Pack size*	Product code
Laboratory pack sizes:		
KappaSelect	25 mL	17545801
KappaSelect	200 mL	17545802
LambdaFabSelect	25 mL	17548201
LambdaFabSelect	200 mL	17548202
Bioprocess pack sizes:		
KappaSelect	1 liter	17545803
KappaSelect	5 liters	17545804
LambdaFabSelect	1 liter	17548203
LambdaFabSelect	5 liters	17548204
Prepacked columns:		
HiTrap KappaSelect	5 × 1 mL	17545811
HiTrap KappaSelect	1 × 5 mL	17545812
HiTrap LambdaFabSelect	5 × 1 mL	17548211
HiTrap LambdaFabSelect	1 × 5 mL	17548212
PreDictor™ plates:		
PreDictor KappaSelect 6	4 × 96-well plates	28980195
PreDictor KappaSelect 20 µL	4 × 96-well plates	28980196
PreDictor KappaSelect 100 µL	4 × 96-well plates	28952733
PreDictor LambdaFabSelect 6 µL	4 × 96-well plates	17548213
PreDictor LambdaFabSelect 20 µL	4 × 96-well plates	17548214
PreDictor LambdaFabSelect 50 µL	4 × 96-well plates	17548215

* Larger resin pack sizes are available. Please contact your local Cytiva representative.

Related literature

KappaSelect; LambdaFabSelect Regulatory Support	File on request
Sofer, G. and Hagel, L. Cleaning, sanitization and storage, in *Handbook of Process Chromatography: A Guide to Optimization, scale-up and validation.* Academic Press, Amsterdam, pp. 188–214 (1997).	18112156
Affinity Chromatography Handbook	18102229
Affinity Columns and Media, Selection Guide	18112186

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