GE Healthcare
Life Sciences

KappaSelect LambdaFabSelect

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 media for purifying kappa and lambda Fab fragments respectively. They enable efficient capture with high purity and yield. Both are part of GE Healthcare Life Sciences' 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

Media 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 respectively (i.e. fragments lacking the constant region of the light chain will not bind; Fig 1). Both are therefore capable of binding other target molecules containing 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 a single-chain antibody fragment that is screened for either human Ig kappa or lambda.

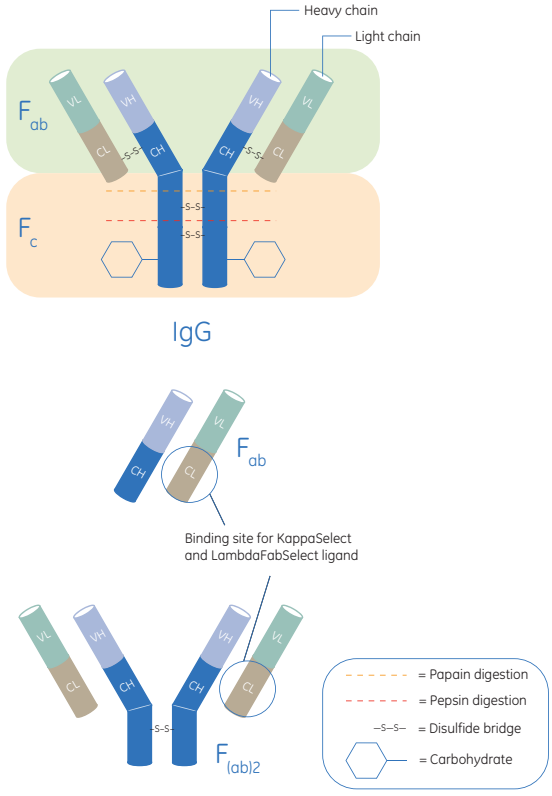


Fig 1. Antibody structure and Fab fragment binding site for KappaSelect and LambdaFabSelect ligand.

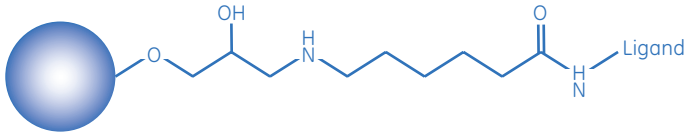


Fig 2. Partial structure of KappaSelect and LambdaFabSelect.

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

Table 1. Main characteristics of KappaSelect and LambdaFabSelect

Matrix	Highly cross-linked high-flow agarose
Particle size*	75 µm (d_{50v})
Ligand	Recombinant protein (M_r 13 000), produced in *S. cerevisiae*, that binds to the constant region of Fab kappa or lambda light chain
Ligand density	Approx. 5 mg/mL medium (KappaSelect)
	Approx. 7 mg/mL medium (LambdaFabSelect)
Binding capacity	Approx. 15 mg Fab/mL medium (KappaSelect)†
	Approx. 20 mg Fab/mL medium (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 < 3 bar (0.3 MPa)
pH stability	
short-term§	2–12
long-term¶	3–10
Working temperature	4°C–30°C
Storage	2°C–8°C, 20% ethanol

* d_{50v} is the mean particle size of the cumulative volume distribution.

† Static capacity for polyclonal Fab kappa determined in 1 mL HiTrap™ column; elution 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 medium can be subjected to cleaning-in-place or sanitization-in-place without significant change in function.

¶ pH interval where the medium can be operated and stored for longer periods of time without significant change in function.

Principles

General affinity chromatography principles exploit an immobilized ligand that adsorbs a specific molecule or group of molecules under suitable binding conditions and desorbs them during suitable elution conditions. These conditions depend on the target molecule, feed composition, and the chromatography medium, and must be studied 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.

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

Equilibration/ washing 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 medium.
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).

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

Application

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 A_{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

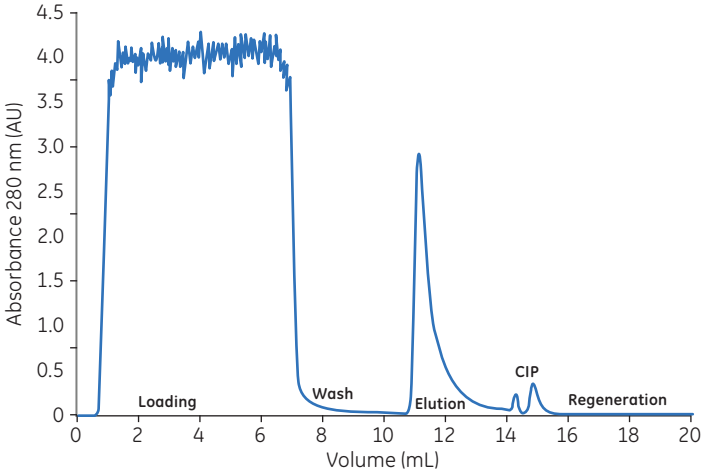
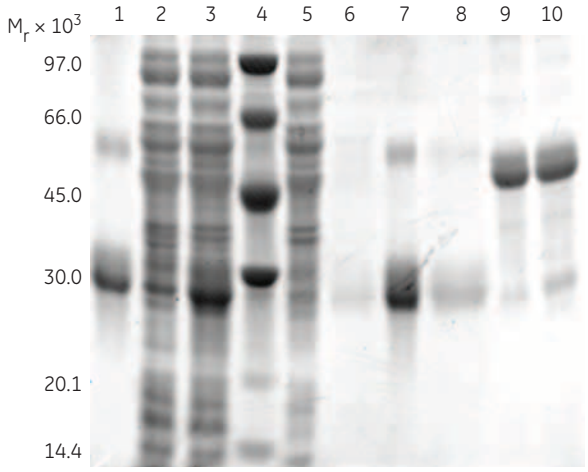


Fig 3. UV_{280} absorbance curve for loading an *E. coli* lysate and eluting human Fab lambda on LambdaFabSelect as the first capture step for lambda Fab purification.



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 Fig. 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 20°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 > 12, both carbon and nitrogen are released, which indicates hydrolysis of the ligand. An identical study has been performed for LambdaFabSelect with similar results.

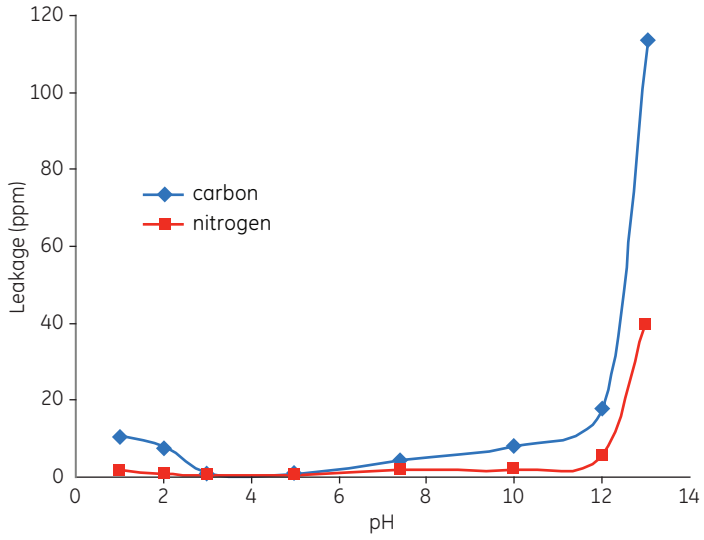


Fig 5. Stability of KappaSelect at different pH.

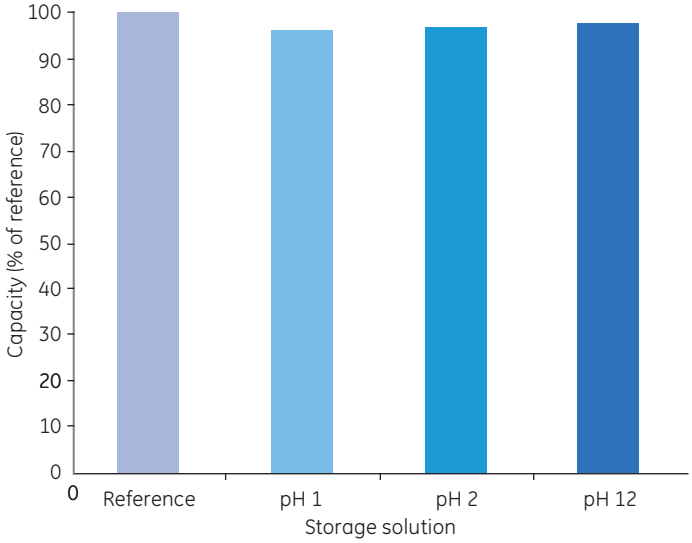


Fig 6. Fab-binding capacity (determined as percent of reference) of KappaSelect after storage in solutions of different pH.

Leakage assay

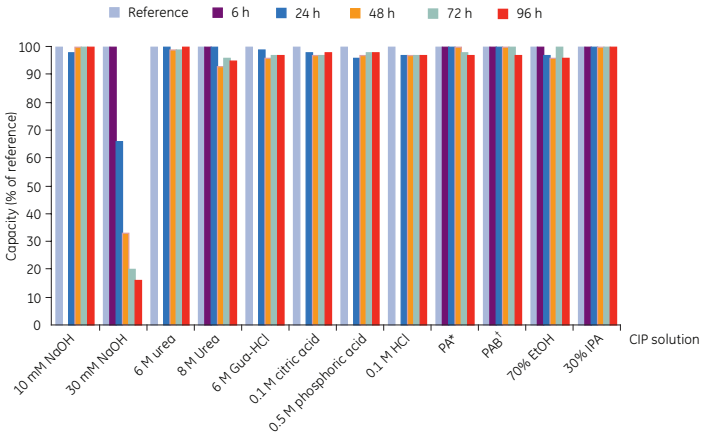
Assays for determination of ligand leakage are available for both products from BAC BV (Bio Affinity Company, Netherlands) through their website (www.captureselect.com/shop).

Cleaning-in-place (CIP) and sanitization

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 (Figs 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. A cleaning or sanitization protocol should be designed for each application since the efficiency of the protocol is strongly related to the feedstock and other related operating conditions.

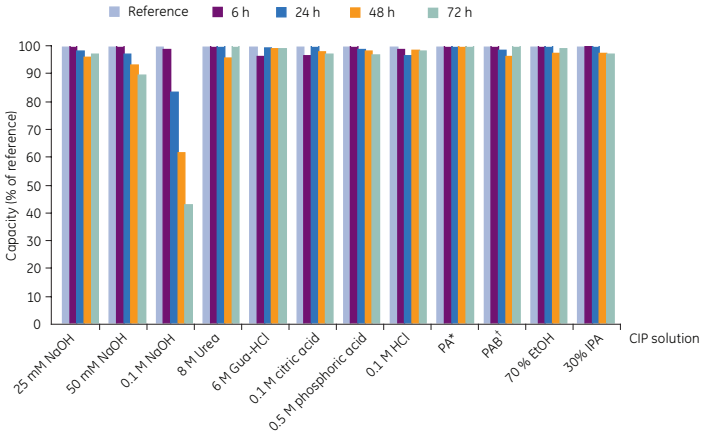
Storage

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



* 120 mM phosphoric acid and 167 mM acetic acid
† PA & 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.

Related literature

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).	18-1121-56
Affinity Chromatography Handbook	18-1022-29
Affinity Columns and Media, Selection Guide	18-1121-86

Ordering information

Product	Pack size*	Code no.
Laboratory pack sizes:		
KappaSelect	25 mL	17-5458-01
KappaSelect	200 mL	17-5458-02
LambdaFabSelect	25 mL	17-5482-01
LambdaFabSelect	200 mL	17-5482-02
Bioprocess pack sizes:		
KappaSelect	1 liter	17-5458-03
KappaSelect	5 liters	17-5458-04
LambdaFabSelect	1 liter	17-5482-03
LambdaFabSelect	5 liters	17-5482-04
Prepacked columns:		
HiTrap KappaSelect	5 × 1 mL	17-5458-11
HiTrap KappaSelect	1 × 5 mL	17-5458-12
HiTrap LambdaFabSelect	5 × 1 mL	17-5482-11
HiTrap LambdaFabSelect	1 × 5 mL	17-5482-12
PreDictor plates:		
PreDictor KappaSelect 6	4 × 96-well plates	28-9801-95
PreDictor KappaSelect 20 µL	4 × 96-well plates	28-9801-96
PreDictor KappaSelect 100 µL	4 x 96-well plates	28-9527-33
PreDictor LambdaFabSelect 6 µL	4 × 96-well plates	17-5482-13
PreDictor LambdaFabSelect 20 µL	4 × 96-well plates	17-5482-14
PreDictor LambdaFabSelect 50 µL	4 × 96-well plates	17-5482-15

* Larger pack sizes of media are available. Please contact your local GE Healthcare Life Sciences representative.

For local office contact information, visit
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