KappaSelect

Antibody fragments, especially Fabs, are getting increased attention as potential biopharmaceuticals because they have some 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 is an affinity medium designed for the purification of Fab (kappa) fragments, enabling an efficient capture step with high purity and yield. KappaSelect is part of GE Healthcare's Custom Designed Media program.

KappaSelect affinity chromatography medium provides:

- 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, which ensures increased Fab purity and productivity

Characteristics of the medium

KappaSelect is based on a highly rigid agarose base matrix that allows high flow rates and low back pressure at large scale. KappaSelect is an affinity medium featuring a ligand that binds to the constant region of the kappa light chain (i.e., fragments lacking the constant region of the kappa light chain will not bind; Fig 1). KappaSelect is therefore capable of binding other target molecules containing the constant region of the kappa light chain, for example, IgA and IgM. The ligand is attached to the matrix via a long hydrophilic spacer arm to make it easily available for binding to the target molecule (Fig 2). The ligand is based on a single-chain antibody fragment that is screened for human Ig kappa. The ligand is produced in a yeast expression system, where fermentation and subsequent purification/formulation is performed in the absence of mammalian components. The characteristics of KappaSelect are summarized in Table 1.

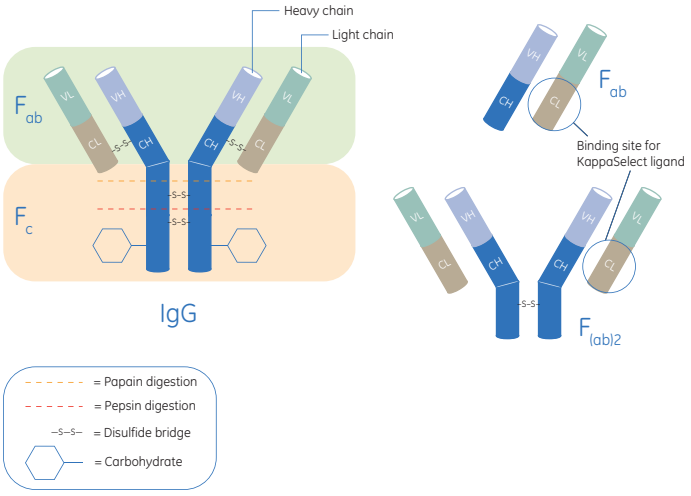

Fig 1. Antibody structure and binding site for KappaSelect ligand to Fab fragment.

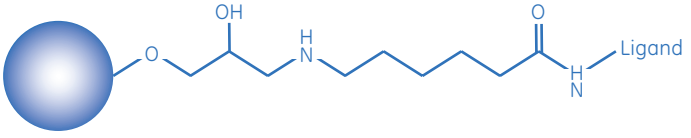

Fig 2. Partial structure of KappaSelect.

Table 1. Main characteristics of KappaSelect

Matrix	Highly cross-linked high-flow agarose
Particle size[1]	75 µm (d_{50v})
Ligand	Recombinant protein (M_r 13 000), produced in *S. cerevisiae,* that binds to constant region of Fab kappa light chain
Ligand density	Approx. 5 mg/ml of medium
Binding capacity[2]	Approx. 11 mg Fab/ml of medium
Flow velocity	At least 600 cm/h in a 1 m 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 long term short term	 3–10 2–12
Working temperature[3]	4°C to 30°C

[1] d_{50v} is the mean particle size of the cumulative volume distribution

[2] Determined using multiwell plates and polyclonal Fab as reagent

[3] Recommended long-term storage conditions: 4°C to 8°C, 20% ethanol

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 these 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, with recommended buffers, 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 3

1. Pack the column with KappaSelect.
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. In order to prevent build-up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place and sanitization).

Stability

The ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low leakage. Figure 3 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 4). At pH values > 12, both carbon and nitrogen are released which indicates hydrolysis of the ligand.

Leakage assay

An assay for determination of ligand leakage is available from BAC BV (Bio Affinity Company, Netherlands) through their website (www.bac.nl).

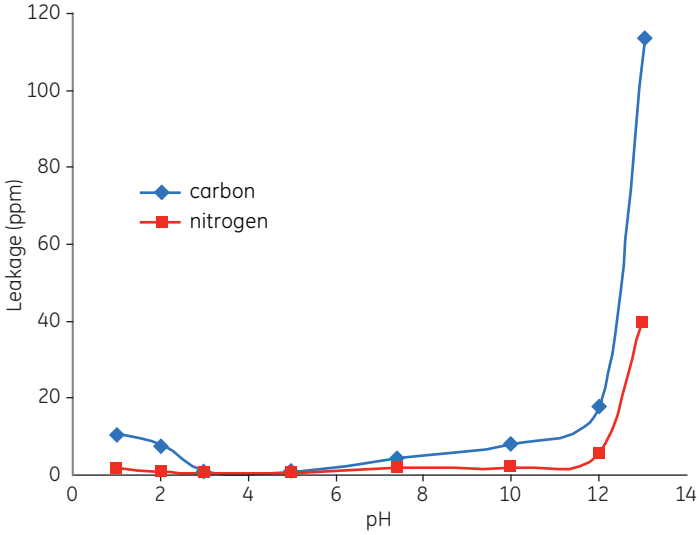

Fig 3. Stability of KappaSelect at different pH.

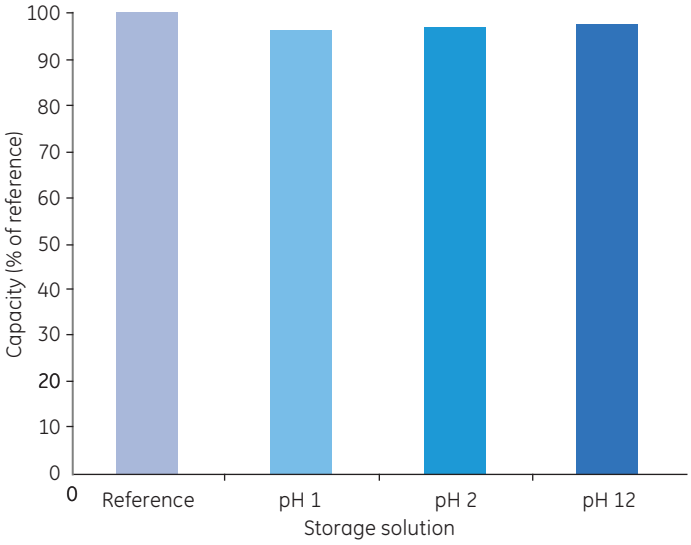

Fig 4. Fab-binding capacity (determined as percent of reference) of KappaSelect after storage in solutions of different pH

Cleaning-in-place (CIP) and sanitization

A study was performed where KappaSelect was treated with various commonly used CIP solutions. The Fab binding capacity was determined after set time intervals (Fig 5). 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. However, prolonged exposure (i.e., several days) to pH < 2 should be avoided due to slow decomposition of the agarose matrix at low pH. A pH > 12 should be avoided due to limited ligand stability under alkaline conditions. A cleaning or sanitization protocol has to be designed for each application.

Storage

We recommend that the medium be stored in 20% ethanol at 4°C to 8°C. KappaSelect is supplied preswollen in 20% ethanol.

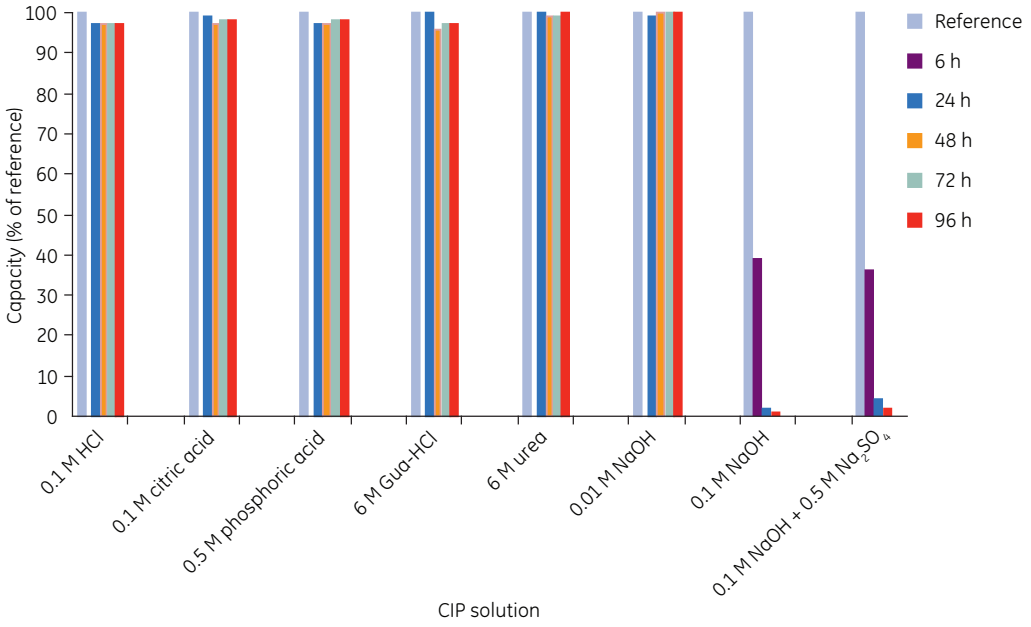

Fig 5. Fab-binding capacity (determined as percent of reference) of KappaSelect after treatment with various CIP solutions.

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
KappaSelect	25 ml[1]	17-5458-01
KappaSelect	200 ml[1]	17-5458-02
KappaSelect	1 l[1]	17-5458-03

[1] Larger pack sizes are available, please contact your local GE Healthcare representative

For contact information for your local office, please visit, www.gelifesciences.com/contact

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