

Single-step affinity purification of antibodies and antibody-fragments by Kappa light chain binding

Pim Hermans, Frank Detmers, Kevin Sleijpen and Jessica de Rooij. Thermo Fisher Scientific, Leiden, the Netherlands

INTRODUCTION

With the development of novel biotherapeutic antibody formats, such as trifunctional and bi-specific monoclonal antibodies or antibody fragments, new purification challenges in the downstream process of these molecules arise. Thermo Scientific™ CaptureSelect™ antibody subdomain-specific affinity products are developed for the discovery and manufacturing of therapeutic antibodies and antibody fragments. The affinity resins provide high target purity in a single step, independent of feedstock.

Here we show the performance of the CaptureSelect KappaXP affinity resin, a next generation Kappa Light chain binder, with improved dynamic binding capacity and mild elution properties, in comparison with alternative products.

CaptureSelect Technology – Unique Affinity Purification Solution

- Affinity through antibody selectivity: technology based on Camelid-derived single domain [V_HH] antibody fragments
- Unique screening technology for target specificity, mild elution & stability
- Animal origin free production process (*Saccharomyces cerevisiae*)
- Technology used in commercial purification processes

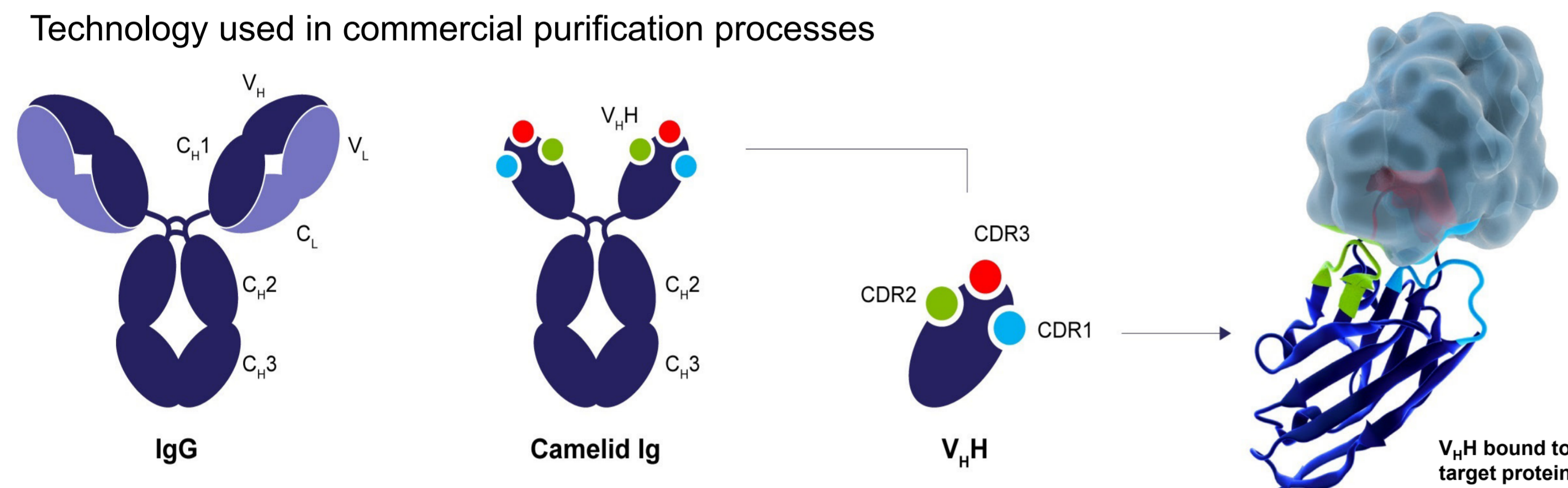
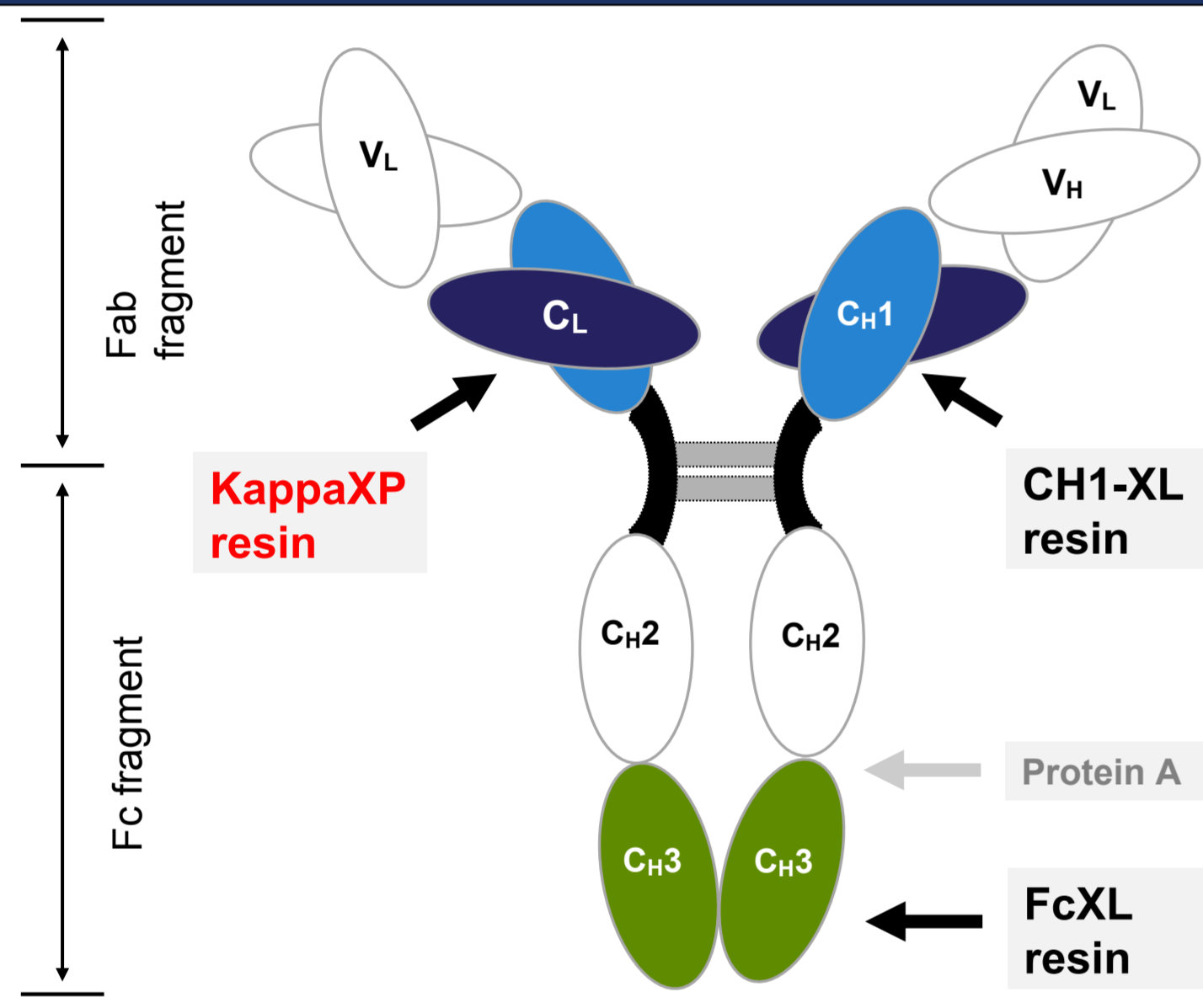


Fig.1 Regular IgG antibody compared to a Camelid heavy-chain antibody. The V_HH antibody fragments offer high specificity, affinity and stability.

CaptureSelect KappaXP: next generation Kappa Light chain binder



- Generic: 100% Kappa subtype coverage for all Ig's containing a Kappa light chain
- Human specific, no binding to bovine antibodies
- High binding capacity:
 - 20-30 g/l Kappa Fab
 - 30-45 g/l IgG
- Mild elution properties (up to pH 6)
- Good stability (75-100 mM NaOH)
- Excellent scalability
- Non-animal-derived

Fig.2 CaptureSelect Antibody Selectivity Binding regions of CaptureSelect resins for affinity purification of antibodies and antibody fragments.

Dynamic Binding Capacity

The CaptureSelect KappaXP resin shows a high binding capacity at 10% breakthrough

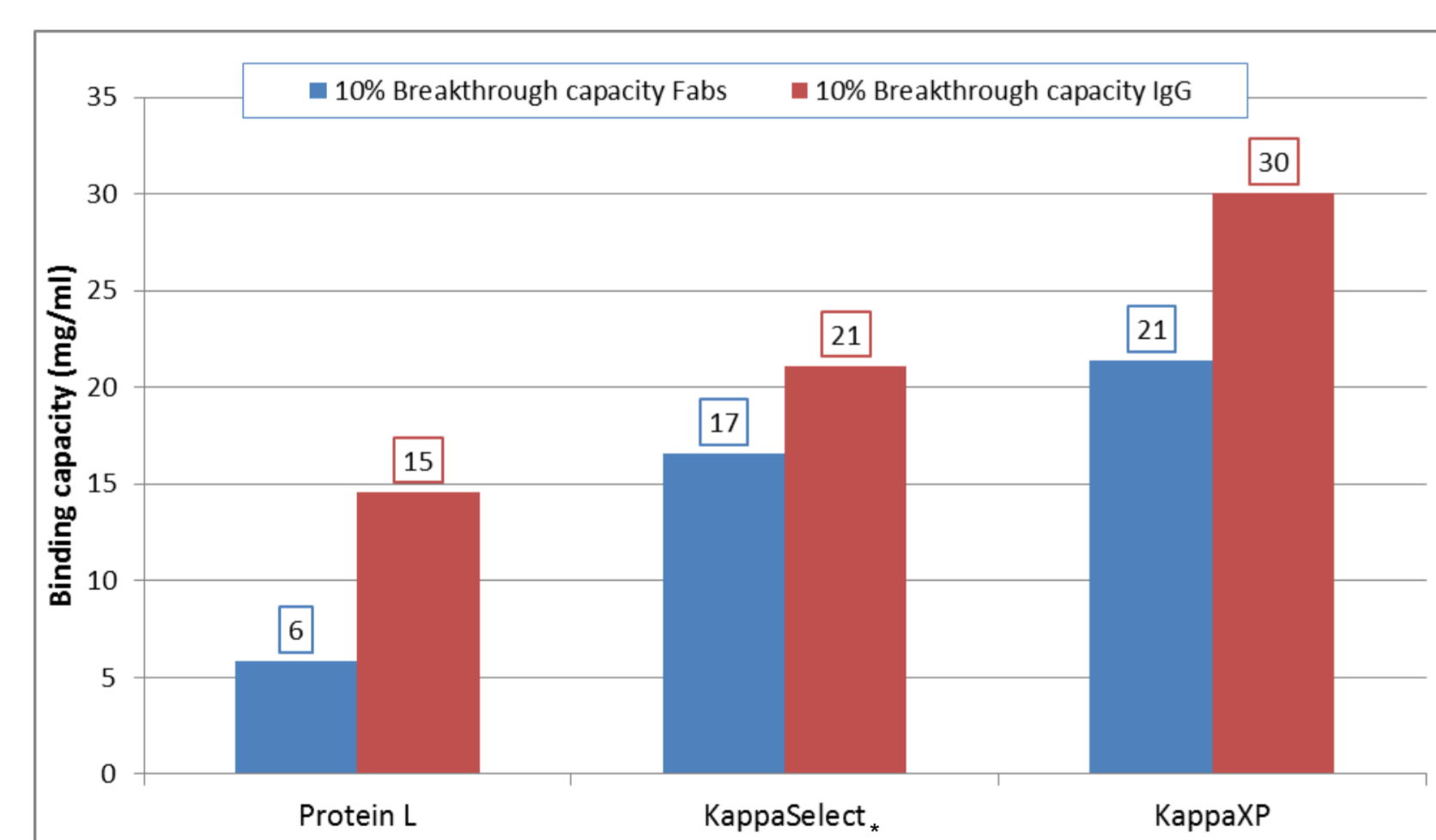


Fig.3 10% breakthrough analysis; comparison with alternative kappa light chain affinity resins.

Capacities measured with Polyclonal IgG and Polyclonal Fab at 6,1 min residence time on 1 min columns. Bound protein eluted using 20 mM Citric Acid pH 3.5.

- Polyclonal human fabs loaded: 62% Kappa, 38% Lambda
- Polyclonal human IgG loaded: 70% Kappa, 30% Lambda

* GE Healthcare

CaptureSelect KappaXP caustic stability

Compared to alternative resins, the CaptureSelect KappaXP resin shows improved caustic stability*

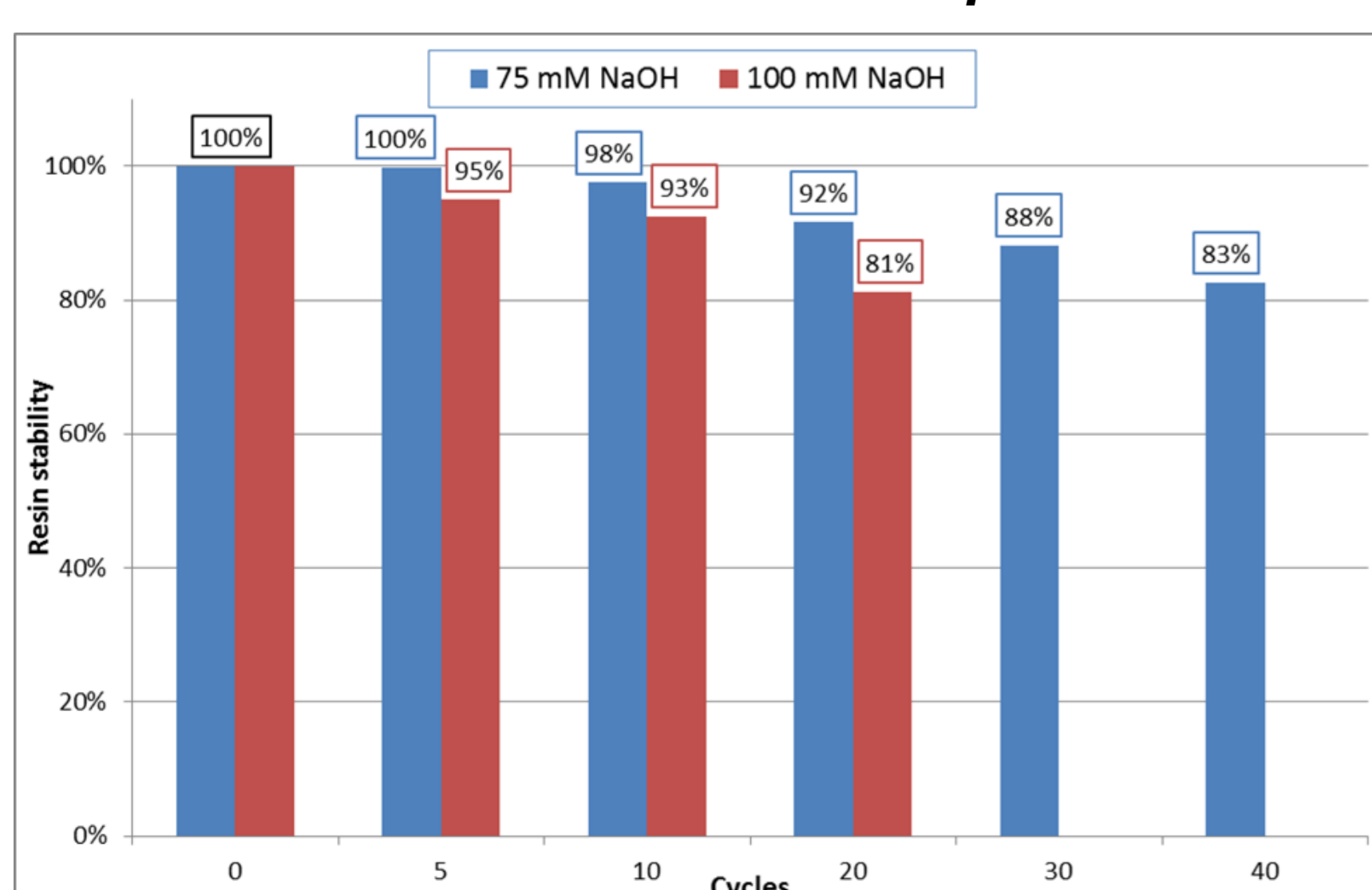


Fig.4 Caustic stability at 75 and 100 mM NaOH

KappaXP resin cycled with 75 mM and 100 mM NaOH

- 15 minutes NaOH exposure,
- 15 minute equilibration with PBS
- Resin capacity measured at different intervals

Capacities measured with Polyclonal IgG at 0,8 min residence time on 400 µl columns. Bound protein eluted using 20 mM Citric Acid pH 3.5.

- 80% capacity left after:
 - 20 cycles with 100 mM NaOH
 - 40 cycles with 75 mM NaOH

* Resin lifetime depends on how the resin is used and cleaned.

Elution efficiency

The CaptureSelect KappaXP resin demonstrates a high elution efficiency, and a large elution operating space (up to pH6 when adding co-solvents)

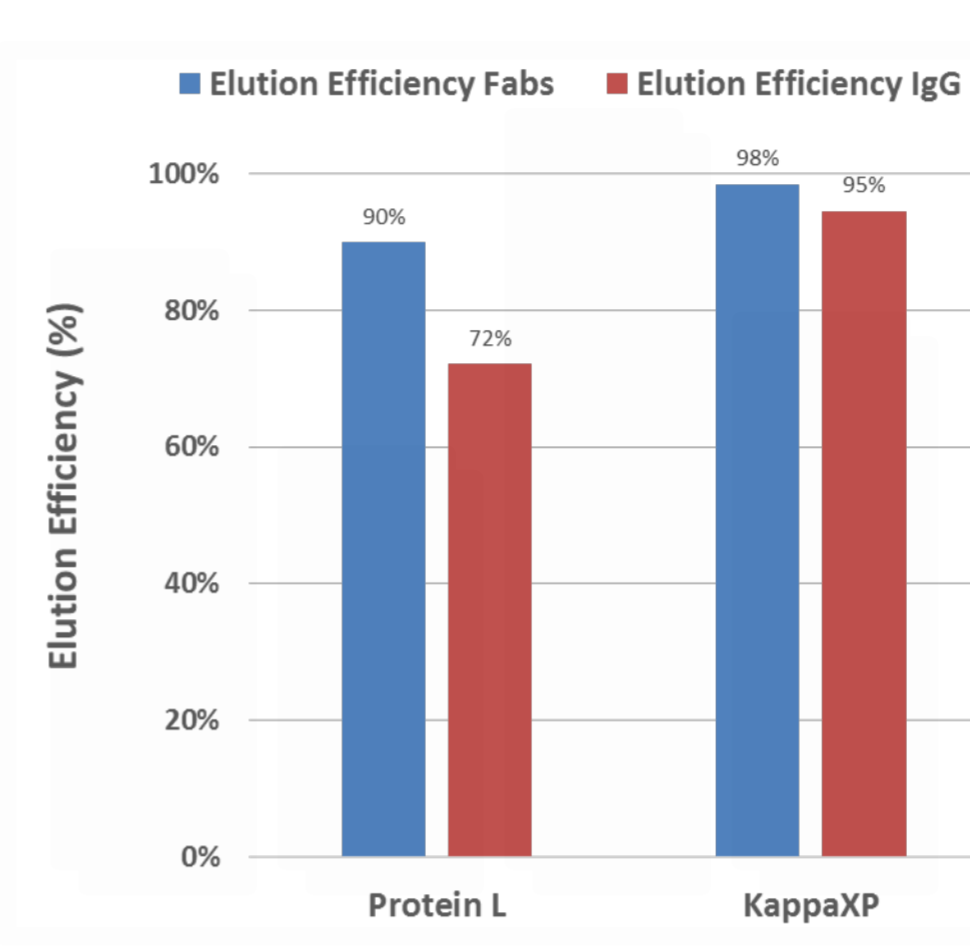


Fig.5 Elution efficiency KappaXP at pH 3.5 compared to Protein L

Elution efficiency determined using polyclonal IgG and polyclonal Fab on 1 ml columns.

Bound protein eluted using 20 mM Citric Acid pH 3.5, at 1.6 min residence time

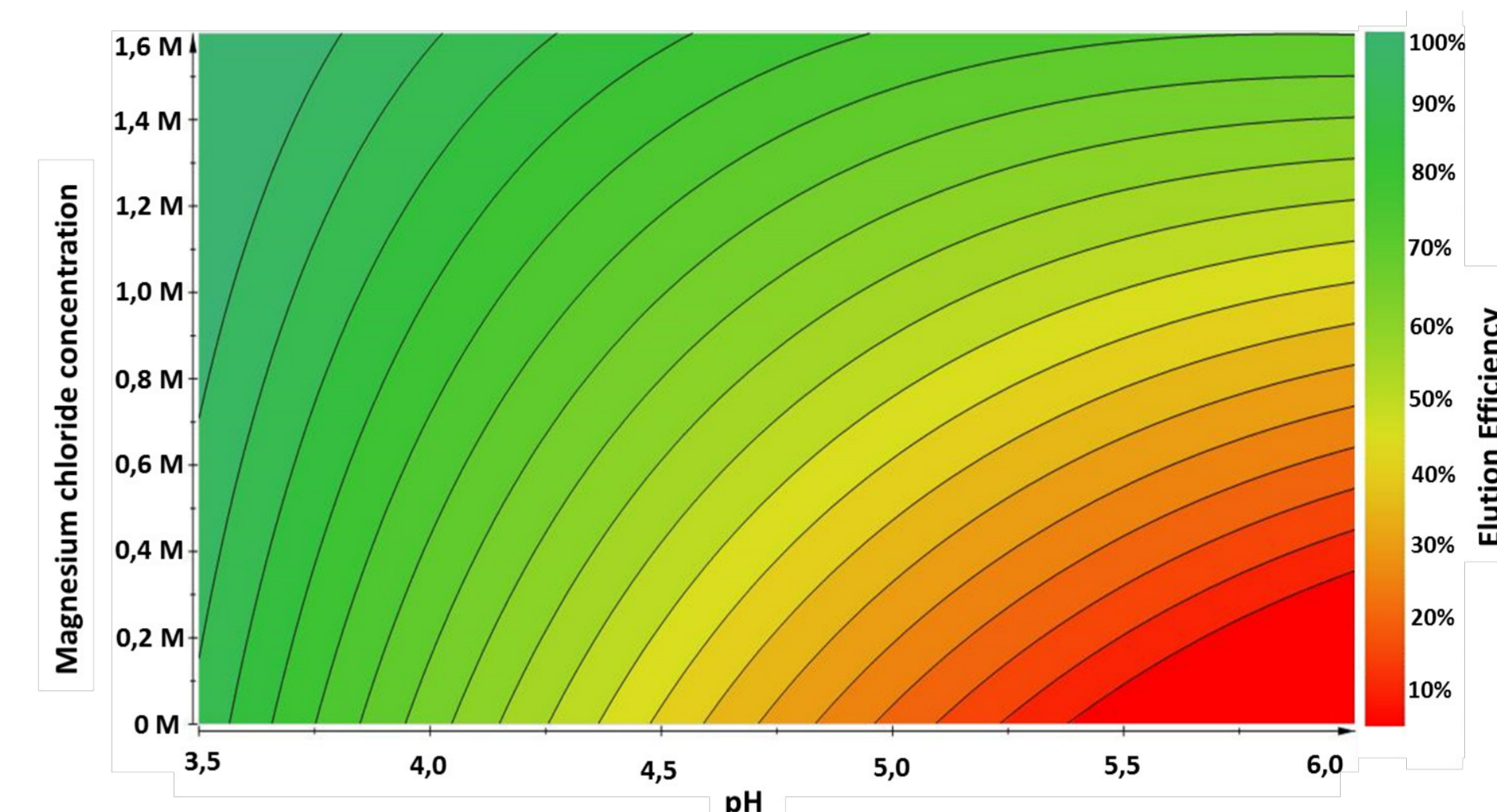


Fig. 6 DOE contour plot elution efficiency KappaXP

Elution efficiency determined using Polyclonal IgG on 200 µl columns. IgG eluted at 0,8 min residence time, 20 column volumes.

>95% elution at pH 5-6 + 1.5 M MgCl₂

Monoclonal Fab and IgG purification examples

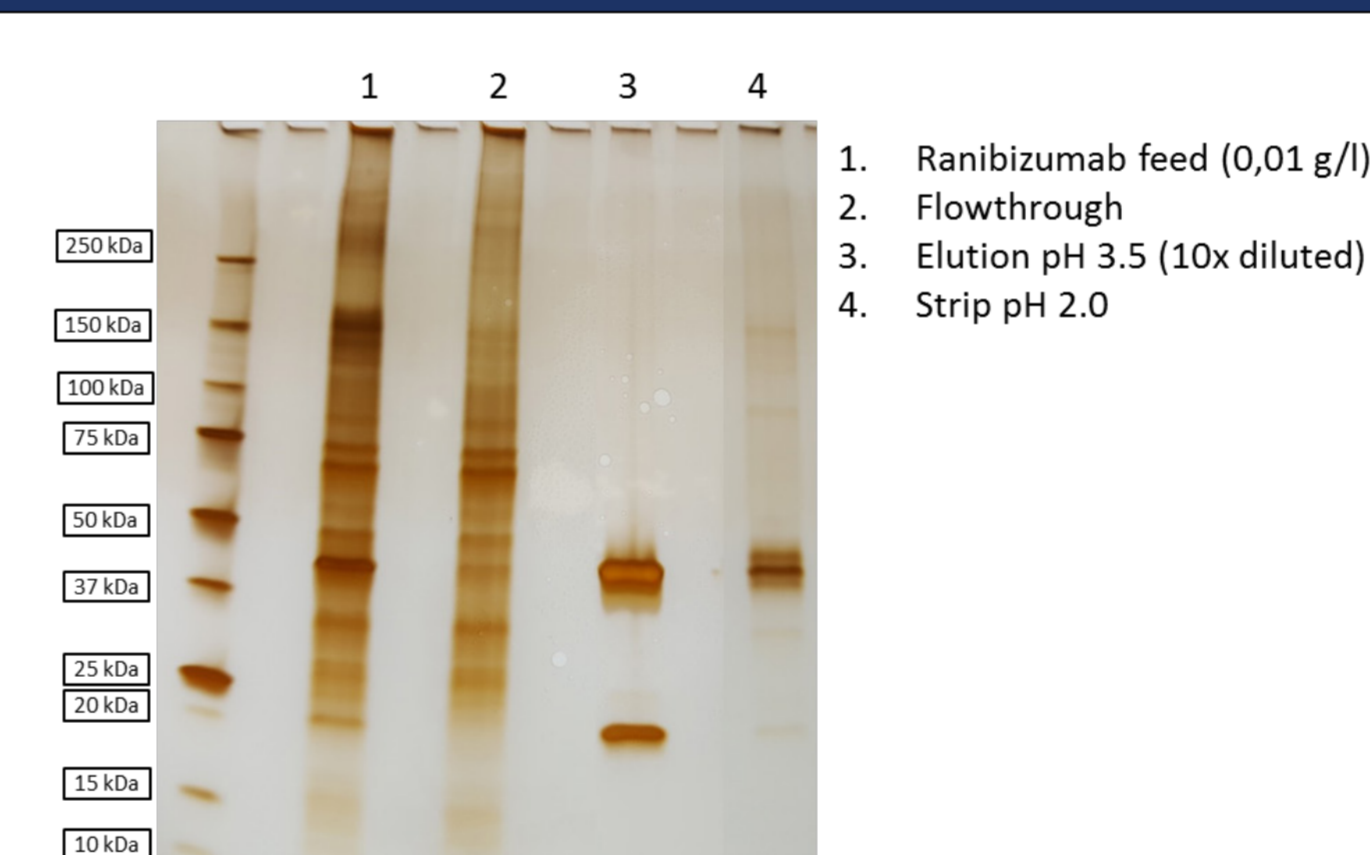


Fig.7 Ranibizumab one-step purification

Purification performed on 1 ml column with 0.5 minutes residence time. Protein was eluted using 20 mM Citric Acid pH 3.5.

- Intact Fab + light chain and light chain dimers present in the elution
- Capacity: 18 g/l

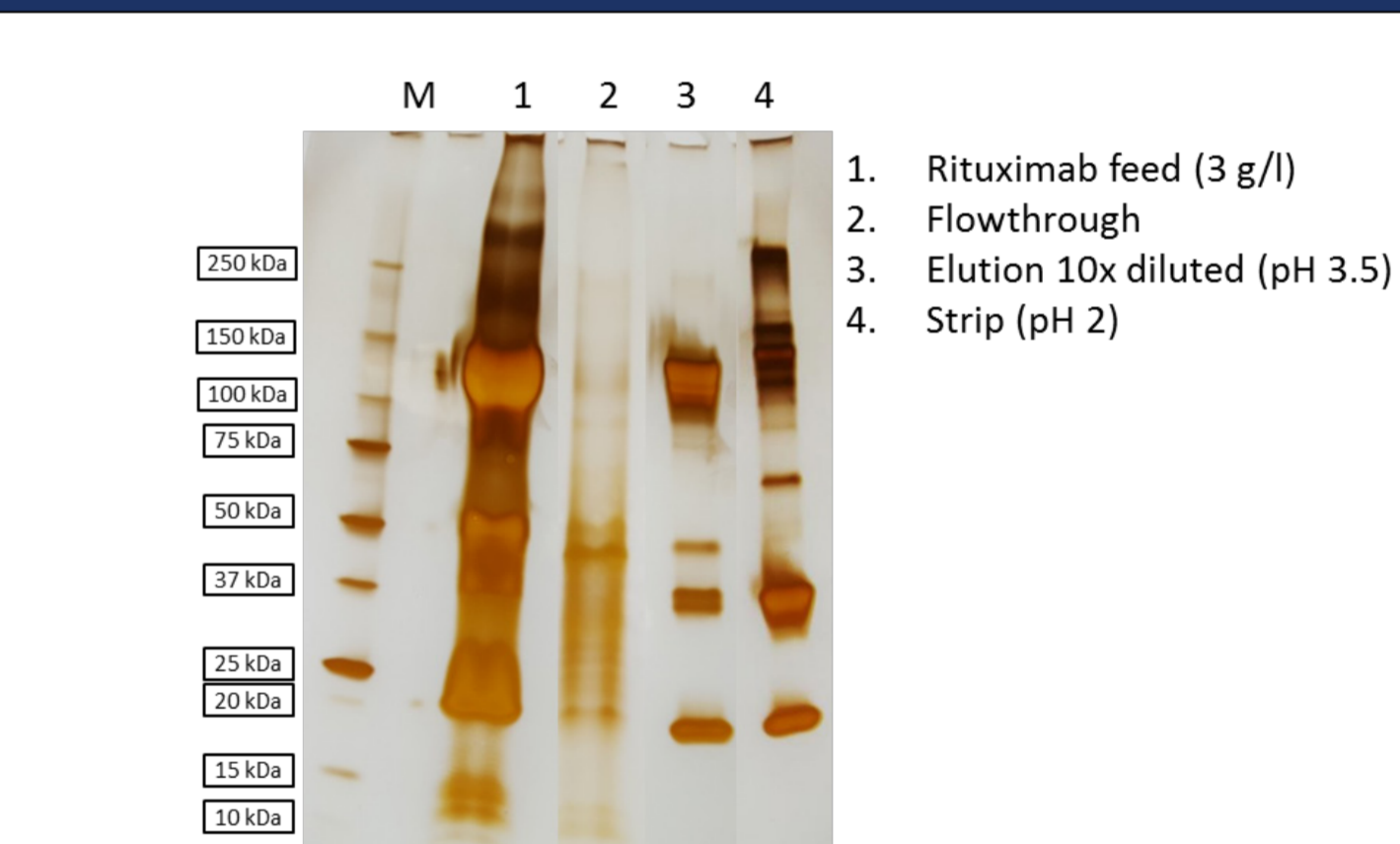


Fig.8 Rituximab one-step purification

Purification performed on 400 µl column with 2 minutes residence time. Protein was eluted using 20 mM Citric Acid pH 3.5.

- Intact IgG + light chain and light chain dimers present in the elution
- Capacity: 45 g/l

Resin characteristics and available products

MAIN RESIN CHARACTERISTICS

Matrix: agarose-based, epoxide activated

Average particle size: 65 ± 10 µm

Ligand: CaptureSelect™ KappaXP affinity ligand

Ligand immobilization method: Epoxide immobilization of the ligand

Fab Binding capacity: 20–30 g IgG Kappa-Fab / liter resin depending on flow rate, column height, and residence time

IgG Binding capacity: 35–45 g IgG / liter resin depending on flow rate, column height, and residence time

Elution conditions: 20 mM citric acid or acetic acid, pH 3–4; 100 mM Tris, 1.5 M MgCl₂, pH 6

Flow characteristics: 150–300 cm/h (up to 2 bar)

Formulation buffer: 20%(v/v) ethanol

SKU	Product
2943212005	CS KappaXP Affinity Matrix - 5 ML
2943212010	CS KappaXP Affinity Matrix - 10 ML
2943212050	CS KappaXP Affinity Matrix - 50 ML
19432120250	CS KappaXP Affinity Matrix - 250 ML*
1943212001L	CS KappaXP Affinity Matrix - 1 L*
1943212005L	CS KappaXP Affinity Matrix - 5 L*
810321201-1	CS KappaXP Leakage Elisa - 1 ASSAY
810321201-10	CS KappaXP Leakage Elisa - 10 ASSAY
5943212005	CS KappaXP MiniChrom™ 5 ML
5943212200	CS KappaXP RoboColumn 200µl
494321205	CS KappaXP COLUMN 1X5 ml
494321201	CS KappaXP COLUMN 5X1 ml

*Products come with full regulatory support (RSF) enabling use in commercial manufacturing MiniChrom is a registered trademark of Repligen



Contact: captureselectsupport@thermofisher.com

Purification of Ig's, bi-specifics, Fab, and Fab2 fragments directly from complex source materials in a single step with high purity and yield.