

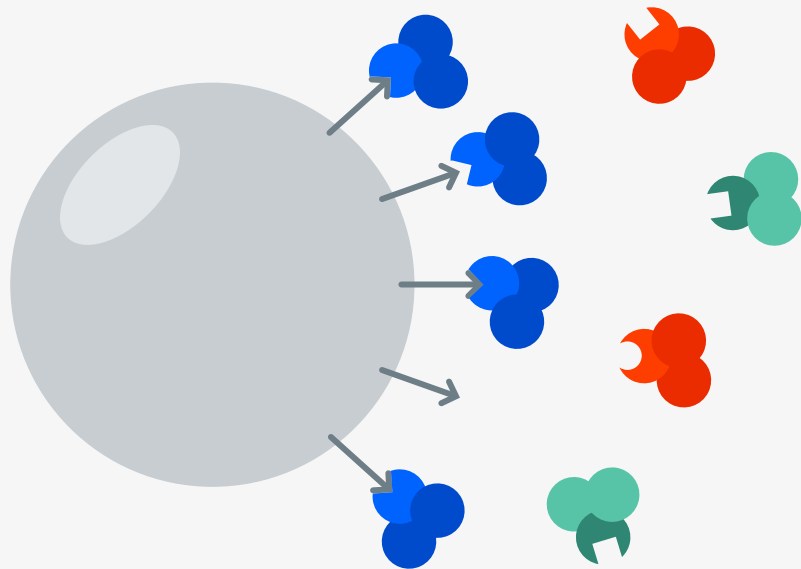
Affinity chromatography

Affinity chromatography (AC) separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatography matrix. The technique is ideal for the capture step in a purification protocol. The target protein is collected in a highly pure and concentrated form. The high selectivity of affinity chromatography enables many purifications to be achieved in only one simple step, for example, purification of antibodies.

Target molecules

Monoclonal and polyclonal antibodies, bound via the Fc-region.

See schematic depicting affinity chromatography.



WorkBeads affimAb

- High dynamic binding capacity at short residence times
- Outstanding alkali stability with 0.5 M NaOH, extends the number of purification cycles
- Excellent purity, recovery and reproducibility
- Negligible protein A leakage
- Available in several different GoBio prepacked columns



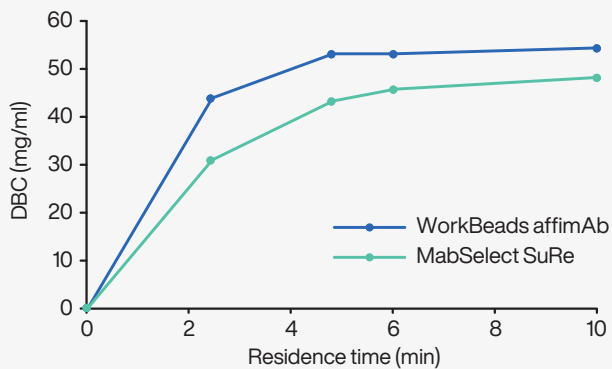
WorkBeads Protein A

- For routine purification of antibodies in the research lab
- High dynamic binding capacity with excellent recovery and purity
- Reliable, reproducible and efficient
- Convenient prepacked 1 mL and 5 mL GoBio Mini columns

Applications

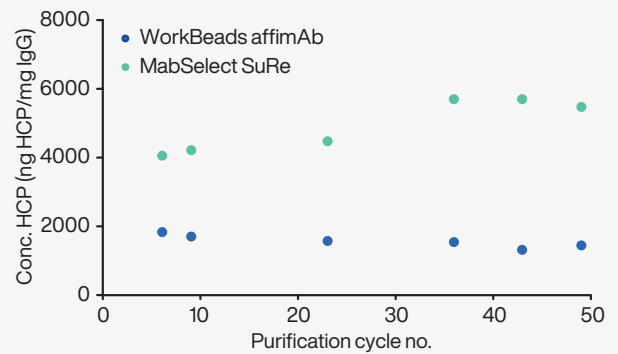
Dynamic binding capacity vs. residence time

Resins:	WorkBeads affimAb MabSelect SuRe™ (Cytiva)
Column volume:	3.4 mL (6.6 × 100 mm)
Sample:	1 mg/mL human polyclonal IgG in PBS, pH 7.4
Binding buffer:	PBS, pH 7.4
Elution buffer:	0.1 M glycine-HCl, pH 2.7
Cleaning-in-place (CIP):	5 column volumes (CV) 0.5 M NaOH at 2.4 min residence time (RT)
Residence times:	2.4, 4.8, 6 and 10 min (250, 125, 100 and 60 cm/h)



HCP and HCD impurities in eluted mAb

Columns:	WorkBeads affimAb: 6.6 × 50 mm, 1.7 mL MabSelect SuRe: 6.6 × 50 mm, 1.7 mL
Sample:	18 mL clarified cell supernatant from CHO cells (100 cm/h)
Binding/wash buffer:	PBS, pH 7.4 (300 cm/h)
Elution buffer:	100 mM glycine-HCl, pH 2.7 (150 cm/h)
CIP:	0.5 M NaOH (100 cm/h), 10 minutes contact time in each cycle
HCP:	CHO CHP ELISA kit (#F550, Cygnus Technologies)
HCD:	Quant-iT™ PicoGreen™ dsDNA Assay Kit (#P7589, ThermoFisher)



Alkali stability comparison

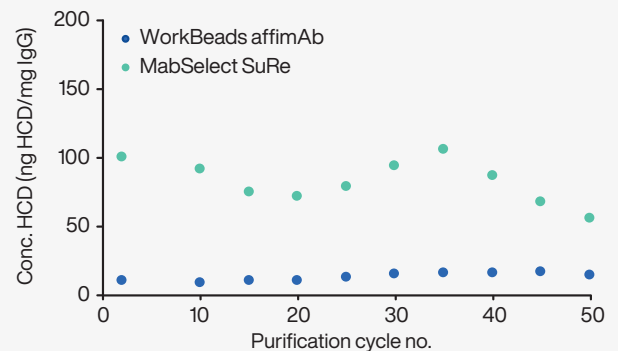
Resins:	WorkBeads affimAb MabSelect SuRe
Column volume:	3.4 mL (6.6 × 100 mm)

DBC (10% breakthrough) determined at start and after each 20th CIP cycle

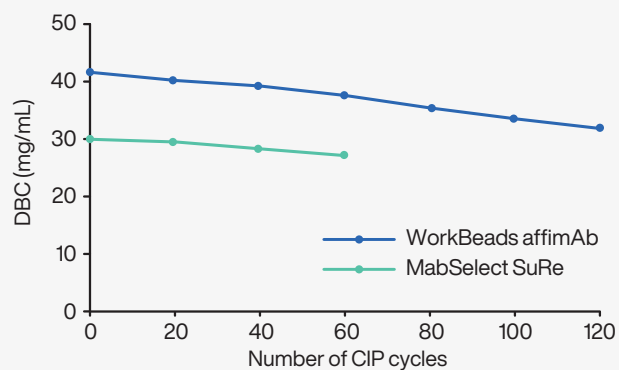
Sample:	1 mg/mL human polyclonal IgG in PBS, pH 7.4
Flow rate:	1.4 mL/min (2.4 min RT)
Binding buffer:	PBS, pH 7.4
Elution buffer:	0.1 M glycine-HCl, pH 2.7

Each CIP cycle:

- 5 CV PBS, pH 7.4 at 1.4 mL/min (2.4 min RT)
- 0.5 M NaOH, 15 min contact time at 1 mL/min
- 5 CV PBS, pH 7.4 at 1.4 mL/min
- 5 CV 0.1 M glycine-HCl, pH 2.7 at 1.4 mL/min
- 5 CV PBS, pH 7.4 at 1.4 mL/min



0.5 M NaOH, 15 min contact time for 120 cycles



Technical specifications

	WorkBeads affimAb	WorkBeads Protein A
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size ¹ (D_{v50})	50 μm	45 μm
Ligand	Alkali stable recombinant protein A expressed in <i>E. coli</i> using animal-free medium	Recombinant protein A expressed in <i>E. coli</i> using animal-free medium
Dynamic binding capacity ² (DBC)	> 40 mg human IgG/mL resin	> 40 mg human IgG/mL resin
Max. recommended flow rate ³	300 cm/h	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 0.5 M NaOH (pH 12), 10 mM HCl (pH 2), 0.1 M sodium citrate-HCl (pH 3), 6 M guanidine-HCl, 20% ethanol Should not be stored at low pH for prolonged time.	Compatible with all standard aqueous buffers used for protein purification, 10 mM HCl (pH 2), 0.1 M sodium citrate-HCl (pH 3), 6 M guanidine-HCl, 20% ethanol, 10 mM NaOH (pH 12) Should not be stored at low pH for prolonged time.
pH stability	3 to 12	3 to 10
Cleaning-in-place (CIP) stability	Up to 0.5 M NaOH	10 mM NaOH
Storage	2 to 8°C in 20% ethanol	2 to 8°C in 20% ethanol

¹ The median particle size of the cumulative volume distribution.

² DBC was determined at 10% breakthrough ($Q_{B10\%}$) by frontal analysis with 1 mg/mL human polyclonal IgG in PBS, pH 7.4 at 1.4 mL/min (245 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb resin, column bed 6.6 × 100 mm.

³ Recommended flow rate at 20°C using aqueous buffers.

Ordering information

Product name	Pack size	Article number
WorkBeads affimAb	25 mL	40 800 001
	200 mL	40 800 002
	1 L	40 800 010
	5 L	40 800 050
	10 L	40 800 060
WorkBeads Protein A	10 mL	40 605 003
	100 mL	40 605 004
	1 L	40 605 005

More information

Data Sheet, DS 40 800 010

WorkBeads affimAb, GoBio prepacked columns

Data Sheet, DS 40 605 010

WorkBeads Protein A, GoBio Mini A

→ bio-works.com/product/affinity-chromatography

