

Lepta rProtein A

1. Basic product information

Lepta rProtein A is an affinity chromatography resin used for the purification of monoclonal and polyclonal antibodies. Recombinant protein A is produced in *E. coli* and engineered for directional conjugation to enhance binding capacity, while maintaining specific binding to the IgG Fc fragment. Epoxy-based coupling ensures low ligand shedding.

2. Chromatography resin parameters

Resin type	Antibody affinity chromatography
Functional group	rProtein A
Matrix	Highly cross-linked agarose
Median particle size	85 µm
Ion load	6 mg rProtein A/ml
Dynamic binding capacity	>35 mg human IgG/ml *
Recommended flow rate	90~500 cm/h
Maximum flow rate	700 cm/h
Maximum working pressure	5 bar
Working temperature	2–40°C

* Measurement conditions of dynamic binding capacity: packing height, 10 cm; retention time, 3 minutes; test buffer, 0.02M NaH₂PO₄ solution, 0.15M NaCl, pH 7.4 when IgG breakthrough reaches 10% of starting concentration.

3. Chemical resistance

pH stability*	3–12
Chemical stability	Common aqueous buffer solution, 30% isopropanol**, 75% ethanol**, 6M guanidine hydrochloride, 8M urea

** The physical and chemical properties and functions of the chromatographic resin have no obvious change after being placed in an environment of 40°C and pH 3–12 for 7 days.*

*** v/v, volume ratio*

4. Method of use

4.1 Chromatographic conditions

1. **Buffer selection:** equilibration/binding/rinse buffer: 20 mM sodium phosphate, 150 mM NaCl, pH 7.0.

Elution buffer: 50 mM sodium citrate, pH 3.0.

1. **Flow rate:** generally choose a linear flow rate of 90–500 cm/h according to the bed height of the column.
2. **Sample pretreatment:** to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 µm microporous membrane before loading it. It is recommended that the pH and conductivity of the sample is adjusted to be consistent with the equilibrium buffer (dilution, ultrafiltration can be used and desalting to adjust the pH and conductivity of the sample).

4.2 Chromatography steps

1. **Equilibration:** use the equilibration buffer to fully equilibrate the chromatography column until the pH and conductivity are stable and basically consistent with the equilibration buffer. This step usually requires 3–5 column bed volumes (CV).
2. **Sample loading*:** according to the binding capacity measured in the small test, determine the sample loading volume and loading amount of the sample on the Lepta rProtein A.
3. **Washing:** use equilibration buffer or other suitable buffer to wash the chromatography column until the UV stabilises and returns to the baseline.
4. **Elution:** elute with lower pH.
5. **Re-equilibration:** re-equilibrate the chromatography column with the equilibration buffer.

5. Cleaning and sterilisation

- To remove strong hydrophobic proteins, lipoproteins and lipids, treat with 0.1% non-ionic detergent at 37°C for 1 minute, then wash with at least 5 CV of binding solution.
- Alternatively soak in 70% ethanol for 12 hours to remove lipids, then rinse with at least 5 CV of binding solution.

Contaminants (such as lipids, endotoxins and proteins) accumulate on the column as the number of uses of the chromatography resin increases. Determine the frequency of cleaning-in-place (CIP) according to the degree of contamination of the chromatography resin (if the contamination is considerable, CIP is recommended after each use to ensure repeatability of results and to prolong the working life of the chromatography resin). For different types of impurities and contaminants, the recommended cleaning conditions are as follows:

- Removal of strongly hydrophobic proteins, lipoproteins and lipids: treat with 0.1% non-ionic detergent at 37°C for 1 minute, then wash with at least 5 CV of binding solution.
- Alternatively soak in 70% ethanol for 12 hours to remove lipids, then rinse with at least 5 CV of binding solution.

To reduce the microbial load, it is recommended that 20% ethanol solution is used to treat the chromatographic resin; treatment time is a minimum of 6 hours.

6. Storage

Keep the unopened chromatography resin in the original container and store at 2~8°C in a well-ventilated, dry and clean place. Do not freeze. Wash the used column with 2–3 CV of 20% ethanol solution and store at 2~8°C.

7. Destruction and recycling

Since chromatography resin is difficult to degrade in nature, it is recommended that the waste chromatography resin is incinerated to protect the environment. For chromatography resin that has been

in contact with biologically active samples such as viruses and blood, follow the local biosafety requirements before destroying or disposing of it.

8. Packing method

Detailed information on resin packaging is available on request. Please contact your local distributor.

9. Ordering information

Product name: Lepta rProtein A

Product Code in Catalogue	Product Volume
652-00025	25 ml
652-00100	100 ml
652-00500	500 ml
652-01000	1 L
652-05000	5 L
652-10000	10 L
652-20000	20 L

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