

Lepta ProtA LX

1. Basic product information

Lepta ProtA LX is an affinity chromatography resin for antibody purification, which separates through the specific interaction between antigen and antibody. This chromatography resin is designed to handle high concentrations of monoclonal antibodies. Lepta ProtA LX has the characteristics of high capacity, high flow rate and alkali resistance:

1. High flow rate and high dynamic binding capacity reduces processing time.
2. The modified alkali-resistant rProtein A ligand can tolerate 0.5–1.0M NaOH for cleaning-in-place (CIP).

2. Chromatography resin parameters

Resin type	Affinity chromatography
Functional group	Alkali-resistant recombinant protein A
Matrix	Highly cross-linked agarose
Median particle size	75 µm
Dynamic binding capacity	>65 mg human IgG/ml *
Maximum flow rate	500 cm/h
Maximum working pressure	5 bar

* Measurement conditions of dynamic binding capacity: packing height, 10 cm; linear flow rate, 100 cm/h; test buffer, 20 mM PB, 0.15M NaCl, pH 7.4 when IgG breakthrough reaches 10% of starting concentration.

3. Chemical resistance

pH stability*	3–12 (working), 2–14 (CIP)
Chemical stability	Common aqueous solution used in protein A chromatography, 10 mM hydrochloric acid, 0.1M sodium citrate, 6M guanidine hydrochloride, 8M urea, 30% isopropyl alcohol**, 20% ethanol**

* 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:** Use neutral buffer as the binding buffer (such as 50 mM PB 0.15 M NaCl, pH 7.0–7.6). Use low pH buffer as eluent (such as 0.1M citric acid, pH 3.0–4.0). Since the ability of Lepta ProtA LX to bind to IgG depends on the source and subtype of antibody, high salt and high pH can promote the binding of antibody and resin. Increase the salt concentration to reduce electrostatic repulsion and enhance the hydrophobic effect. For different antibodies, the binding conditions and washing conditions can be optimised by changing the salt type, concentration and pH of the buffer. When optimising the elution conditions, it is necessary to explore the maximum pH for effective desorption to avoid denaturation of unstable antibodies when the pH is too low.
2. According to the height of the column, a linear flow rate of 60~500 cm/h is generally selected. The higher the column bed height, the slower the flow rate.
3. **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 equilibration 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 ProtA LX.
3. **Rinse:** after loading, the UV absorption is reduced to the appropriate value by the balance buffer. If necessary, high salt or slightly lower pH can be added to clean the non-specific adsorption impurities as much as possible.
4. **Elution:** a linear gradient of 10 CV from equilibration solution to elution buffer (such as 1M sodium citrate, pH 3.0) can be used to determine the optimal pH of elution according to the peak position of the antibody. If the antibody is unstable under acidic conditions, the eluent can be neutralised with a neutralising solution (such as 1.0M Tris-HCl, pH 9.0).
5. **Re-equilibration:** re-equilibrate the chromatography column with the equilibration buffer.

5. Cleaning and sterilisation

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 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). Recommended cleaning conditions are as follows:

- First wash the column with 2~3 CV of binding buffer.
- Then wash the column with 0.1–0.5M NaOH; contact time is 10–15minutes.

Note: Lepta ProtA LX can tolerate 0.5~1.0M NaOH. NaOH concentration, contact time and contact frequency will all affect the cleaning effect. High concentration and long duration can increase the CIP

effect, but correspondingly, it will accelerate the decreasing trend of IgG's binding capacity, so generally choose 0.1~0.5M NaOH. If the pollution is severe, 0.5~1.0M NaOH can be selected.

- Immediately flush with at least 5 CV of binding buffer.

Note: If the antibody bound to the resin is not completely eluted, regeneration should be performed prior to CIP. Before performing CIP with NaOH, it is recommended that the column is equilibrated with a neutral pH solution to avoid direct contact between the low pH buffer and the high pH NaOH solution, which may increase the temperature inside the column.

Since the 20% ethanol preservation solution does not have the effect of sterilisation and depyrogenation, it is recommended that the Lepta ProtA LX resin is treated with 0.5M NaOH for 15~30 minutes before and during use to reduce the risk of microbial contamination and pyrogen.

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 ProtA LX

Product Code in Catalogue	Product Volume
150-00025	25 ml
150-00100	100 ml
150-00500	500 ml
150-01000	1 L
150-05000	5 L
150-10000	10 L

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