

Lepta Butyl

1. Basic product information

Lepta Butyl is based on high-rigidity agarose with weakly hydrophobic aliphatic butyl groups. It has low back pressure and fast flow rate and is suitable for large-scale separation and purification of highly hydrophobic biomolecules. This series of chromatographic resin can be used effectively for the separation and purification of various biological molecules, such as recombinant proteins, antibodies, viruses and vaccines.

2. Chromatography resin parameters

Resin type	Hydrophobic interaction
Functional group	Butyl
Matrix	Highly cross-linked agarose
Median particle size	80 µm
Ligand density	~53 µmol butyl/ml
Dynamic binding capacity	27 mg BSA/ml
Recommended flow rate	150–350 cm/h
Maximum flow rate	1200 cm/h
Maximum working pressure	5 bar
Working temperature	4–30°C

3. Chemical resistance

pH stability	2–14 (cleaning-in-place – CIP) 3–13 (working)*
Chemical stability	All commonly used aqueous buffers, 1M NaOH**, 1M acetic acid**, 6M guanidine hydrochloride, 30% isopropyl alcohol, 70% ethanol

* The physical and chemical properties and functions of the chromatographic resin did not change significantly after being placed in an environment of 40°C and pH 3–13 for 7 days.

*** suitable for CIP only*

4. Method of use

4.1 Chromatographic conditions

1. **Buffer selection:** the binding buffer is usually a phosphate buffer containing a high concentration of salt, such as 20 mM PB, 1.5-2M $(\text{NH}_4)_2\text{SO}_4$, pH 7.0. The elution buffer normally uses phosphate buffer without other salts, such as 20-50 mM PB, pH 7.0, which needs to be based on the subsequent experimental results (whether there is precipitation of the target, binding strength of the target, recovery rate, resolution, etc.) Adjust the concentration and type of salt in the binding buffer. For substances that are difficult to elute, pure water can be used, or lowconcentration ethanol can be added to the pure water as the eluent.
2. **Flow rate:** generally, choose a linear flow rate of 150–350 cm/h according to the column bed height.
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 are 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 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 Lepta Butyl.
3. **Impurity washing*:** use equilibration buffer or other suitable buffer to wash the chromatography column until the UV stabilises and returns to the baseline.

4. **Elution***: elution is achieved by decreasing the concentration of salt ions. The concentration of salt ions in the elution buffer can be gradually decreased through a linear gradient or a step gradient to elute molecules with different binding strengths. Fraction collection was performed on the eluted samples. pH gradient elution or mixed elution can also be used.
5. **Regeneration**: rinse the column with a buffer containing low salt.
6. **Re-equilibration**: re-equilibrate the column with equilibration buffer.

** Note: If the flow-through mode is used, the 'sample loading' step should be set to collect; the 'washing' step should ensure that the target molecules have completely flowed through, then the collection can be stopped; the 'elution' step should directly use low-salt buffer – impurities can be washed away.*

5. Cleaning and regeneration

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). For different types of impurities and contaminants, the recommended cleaning conditions are as follows:

- **Removal of strongly binding proteins**: wash with 5 CV of ultrapure or pure water.
- **Removal of strongly hydrophobic proteins and precipitated proteins**: first wash with 5 CV of 1M NaOH solution, then wash the lye with 5–10 CV of ultrapure or pure water.
- **Removal of lipoproteins and lipids**: first wash with 5 CV of 70% ethanol or 30% isopropanol, then rinse with 5–10 CV of ultrapure or pure water.

Note: 70% ethanol or 30% isopropanol should be degassed before use; the flow rate should be 30–60 cm/h during CIP. Reverse cleaning should be used when the clogging is severe.

To reduce the microbial load, it is recommended that 0.5~1M NaOH solution is used to treat the chromatographic resin. Treatment time is 15~30 minutes.

6. Storage

Keep the unopened chromatography resin in the original container and store at 4~30°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 Butyl

Product Code in Catalogue	Product Volume
133-00025	25 ml
133-00100	100 ml
133-00500	500 ml
133-01000	1 L
133-05000	5 L
133-10000	10 L
133-20000	20 L

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