Capto™ Lentil Lectin

AFFINITY CHROMATOGRAPHY

Capto™ Lentil Lectin is an affinity chromatography resin for purification of glycoproteins and other molecules containing carbohydrates such as α-D-mannose and α-D-glucose or sterically related residues. The product is based on a rigid high-flow agarose base matrix that enables high flow rates. Capto™ Lentil Lectin resin is available in bulk as well as in various formats for high-throughput process development (Fig 1).

Key features of Capto™ Lentil Lectin include:

- Group-specific adsorbent for carbohydrate-containing molecules
- High productivity and cost-efficiency in downstream operations
- Animal component-free production
- Security of supply and regulatory support

Characteristics

Lectins are proteins that interact specifically and reversibly with certain carbohydrate residues. Immobilized lectins are valuable tools for isolating and separating glycoproteins, glycolipids, polysaccharides, subcellular particles, and for purifying detergent-solubilized cell membrane components. Lectins are also useful for assessing changes in levels or composition of surface glycoproteins during cell development and in malignant or virally transformed cell variants.

Capto™ Lentil Lectin resin is designed by coupling lentil lectin to an N-hydroxysuccinimide (NHS)-activated high-flow agarose base matrix (Fig 2). Lentil lectin is a metalloprotein isolated from *Lens culinaris* (lentil) seeds and binds to molecules containing α-D-mannose, α-D-glucose, or sterically related residues. To maintain the binding characteristics of Capto™ Lentil Lectin, the presence of both Mn^{2+} and Ca^{2+} is essential. These ions are present in large excess in the recommended storage solution for the resin. The lectin-metal ion complex remains active and is stable at neutral pH, even in the absence of free metal ions. However, to preserve the binding activity of the resin at pH < 5, excess Mn^{2+} and Ca^{2+} (1 mM) should be present in the buffer solutions. Main characteristics of Capto™ Lentil Lectin are summarized in Table 1.

Fig 1. Capto™ Lentil Lectin resin is produced in an animal component-free environment and is covered by our security of supply program.

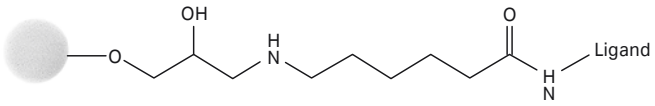

Fig 2. Schematic structure of Capto Lentil Lectin resin.

Table 1. Main characteristics of Capto™ Lentil Lectin resin

Matrix	Highly cross-linked agarose, spherical
Average particle size (d_{50v})[1]	75 µm
Ligand	Lentil (*Lens culinaris*) lectin
Ligand concentration	3 g/L
Coupling chemistry	NHS
Total binding capacity[2]	~ 15 mg porcine thyroglobulin/mL resin
pH stability, operational and CIP[3]	3 to 10
Chemical stability	Stable in commonly used aqueous buffers
Avoid	Chelating agents (EDTA, 8 M urea, or solutions with pH < 3)[5]; contact between storage and NaOH solutions[6]
Flow, recommended operating[4]	100 to 300 cm/h
Flow, maximum[4]	300 cm/h
Storage	2°C to 8°C in 20% ethanol containing 150 mM NaCl, 1 mM $CaCl_2$ and 1 mM $MnCl_2$

[1] Median particle size of the cumulative volume distribution.
[2] We incubated porcine thyroglobulin with the resin in 0.1 M phosphate buffer, pH 7.0, for 3 h in a 10 mL tube, then separated the resin from the solution by centrifugation. The binding capacity was calculated by determining the absorbance of the solution at 280 nm and comparing it to a standard sample.
[3] pH range where the resin can be operated (operational) or be subjected to cleaning- or sanitization-in-place (CIP) without significant change in function.
[4] 20 cm bed height, determined with water at 20°C. Operating pressure is < 0.2 MPa (< 2 bar, 29 psi).
[5] These conditions result in removal of manganese from lectin, leading to loss of activity of the resin.
[6] NaOH and manganese react to form $Mn(OH)_2$ precipitate, which can discolor the resin, and have a negative effect on capacity and backpressure over time.

Operation

Binding

Binding of glycoproteins and other carbohydrate-containing molecules to Capto™ Lentil Lectin resin occurs at a neutral pH in the presence of both Mn^{2+} and Ca^{2+}. These ions are present in excess in the solution in which the resin is supplied. The protein-metal ion complex remains active and is stable at neutral pH even in the absence of the free metal ions. However, to preserve the binding activity at pH < 5, excess Mn^{2+} and Ca^{2+} (1 mM) is required. Recommended binding buffer is 20 mM Tris-HCl, pH 7.4 containing up to 0.5 M NaCl to avoid non-specific ionic interactions.

Elution

Elution of bound substances can be achieved using an increasing gradient (continuous or step) of α-D-methylmannoside or α-D-methylglucoside. These carbohydrates act as strong eluents and many substances elute at 0.1 to 0.2 M. Higher concentrations might be required for more tightly bound substances. Glucose and mannose may also be used, but are weaker eluents. Strongly bound substances can also be eluted using low pH (within operating range) or with a 0.1 M borate buffer, pH 6.5. Elution of strongly bound substances can be facilitated by including 1% deoxycholate or other detergent in the elution buffer.

Regeneration

Capto™ Lentil Lectin can be regenerated by washing the resin with two to three bed volumes of a buffer solution containing 0.5 M NaCl, alternately with high pH (8.5) and low pH (5.5) between wash cycles. These cycles should be repeated three times followed by re-equilibration with three to five bed volumes of binding buffer. All strongly bound substances might not elute during the regeneration procedure. In such cases, a borate buffer containing 0.1% non-ionic detergent could be used at a low flow rate. A 20% ethanol wash or a gradient wash with up to 50% ethylene glycol may also be used to elute strongly bound substances.

As an alternative regeneration method, the resin can be washed with a detergent solution (e.g., 0.1% Triton™ X-100) at 37°C for 1 min. Re-equilibrate with at least five bed volumes of binding buffer after regeneration.

Chemical stability

The chemical stability of Capto™ Lentil Lectin was determined by a total organic carbon/nitrogen (TOC/TN) leakage analysis after storage in buffers of various pH for one week at 40°C (Fig 3). The results show that Capto™ Lentil Lectin is stable between pH 3 and 10. At a pH < 3 or > 10, the leakage of both carbon nitrogen increases.

It is common to perform cleaning-in-place (CIP) or sanitization-in-place of tubing and equipment with NaOH prior to a chromatography run. If there is NaOH remaining in the system or tubing when buffers including $MnCl_2$ or $CaCl_2$ are added to the system, $Mn(OH)_2$ will precipitate and discolor the solutions. As with accumulation of most types of particle, this can cause a negative effect on capacity and backpressure over time. Hence, it is important to remove NaOH from the hardware or tubing before connecting the column and applying 1 mM $MnCl_2$ or $CaCl_2$ buffer for Capto™ Lentil Lectin runs. One way to do this is to rinse the system with a buffer that can neutralize remaining NaOH before adding $MnCl_2$ or $CaCl_2$ buffer. It is also important to increase the buffer pH with NaOH before adding $MnCl_2$ to avoid precipitation and discoloration from $Mn(OH)_2$.

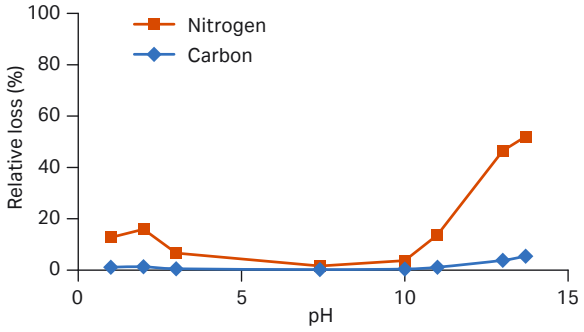

Fig 3. Relative loss of carbon and nitrogen from Capto™ Lentil Lectin resin when stored in different pH for one week at 40°C.

Storage

Capto™ Lentil Lectin is supplied preswollen as a suspension in 20% ethanol containing 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ (storage solution). Recommended storage is at 2°C to 8°C in storage solution.

Ordering information

Product	Quantity	Product code
Capto™ Lentil Lectin	25 mL	17548901
Capto™ Lentil Lectin	100 mL	17548902
Capto™ Lentil Lectin	1 L	17548903
Capto™ Lentil Lectin	5 L	17548904
HiTrap™ columns with Capto™ Lentil Lectin	5 × 1 mL	17548911
HiTrap™ columns with Capto™ Lentil Lectin	1 × 5 mL	17548912
HiScreen™ columns with Capto™ Lentil Lectin	2 × 4.7 mL	29157958

Related literature	Product code
HiScreen™ prepacked columns, data file	CY13473
Affinity Chromatography Handbook, Vol. 3: Specific Groups of Biomolecules	CY13979

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