
NMab ProtL

Protein L Affinity Resin

Product Instruction

Document No. : NM-W-DF-0316

Version: A1

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Protein L affinity resin

NMab ProtL is designed for purification antibody fragments which incorporate the kappa light chain. It is an affinity resin prepared by immobilizing recombinant protein L ligands onto a rigid highly cross-linked agarose matrix through epoxy activation. Protein L exhibits a potent affinity towards the variable region of the antibody kappa light chain.

NMab ProtL is widely used for capture of antibody fragments such as Fabs, single domain antibodies (dAbs), and single-chain fragment variables (scFv), etc.

Table 1. Characteristics of NMab ProtL

Product name	NMab ProtL
Chromatography technique	Protein L affinity
Matrix	Highly cross-linked agarose
Coupling chemistry	Epoxy activation
Dynamic binding capacity	~75 mg·mL ⁻¹ (Fab, 5 min retention time)
Maximum Pressure	0.3 MPa
CIP	6 M guanidine hydrochloride
Recommended flow rate	100-500 cm/h
pH stability	2-10
Chemistry stability	Stable in commonly used buffers, 20 mM sodium phosphate, 1% SDS, 6 M guanidine hydrochloride, 70% ethanol, 6 M urea, etc. Avoid long-term exposure to strong acids or strong bases.
Storage	2-8 °C, 20% Ethanol

Table 2. Affinity capacity of protein L with respect to different species of antibodies.

Species	Antibody Class	Affinity
General	Kappa light chain	Strong
	Lambda light chain	No binding
	Heavy chain	No binding
	Fab	Strong
	ScFv	Strong
	Dab	Strong
Human	IgG1	Strong
	IgG2	Strong
	IgG3	Strong
	IgG4	Strong
	IgA	Strong
	IgD	Strong
Mouse	IgE	Strong
	IgM	Strong
	IgG1	Strong
	IgG2a	Strong
	IgG2b	Strong
	IgG3	Strong
Rat	IgM	Strong
	IgG1	Strong
	IgG2a	Strong
	IgG2b	Strong
Pig	IgG2c	Strong
	Total IgG	Strong
Dog	Total IgG	Weak
Cow	IgG1	No binding
	IgG2	No binding
Goat	IgG1	No binding
	IgG2	No binding
Sheep	IgG1	No binding
	IgG2	No binding

Species	Antibody Class	Affinity
Chicken	Total IgG	No binding

Instructions

Slurry concentration determination

NMab ProtL resin is originally supplied in 20 % ethanol at a slurry concentration (Cs) of approximately 65% (v/v). The term “slurry concentration” denotes the ratio of the constant settling volume of the resin to the total volume of the slurry. To achieve a precise measurement of the slurry concentration, resuspend the resin by gentle agitation in the original container. Subsequently, extract a 10mL sample of the slurry, place it into a measuring cylinder, and allow it to stand overnight. Following this resting period, read the settling volume Vr, and calculate the slurry concentration by the following formula:

$$Eq. 1 \quad C_s (\%) = 100 \times (V_r/10) = 10 V_r$$

To achieve the optimal column packing, it is recommended to prepare 50-70 % of the slurry with 0.5 M NaCl solution.

Packing Preparation

- 1) Calculate the column volume (Vc) of the loaded column

$$Eq. 2 \quad V_c = h \times \pi r^2$$

Where,

h is the column height;

r is the column radius

- 2) Calculate the required slurry volume (Vs)

In general, the resin will be compressed under pressure, resulting in volume shrinkage. To ensure the formation of a compact column bed,, it is recommended that the resin volume is prepared in excess, generally with a compression factor (CF) of 1.15-1.2.

$$Eq. 3 \quad V_s = 100 \times (V_c \times CF)/C_s$$

- 3) Slurry preparation for column packing

Resuspend the resin in the original container, measure the required volume of the original solution Vs into a suitable container, allow the resin particles to settle naturally, then tilt the container to pour off the supernatant; use a packing buffer, such as 0.5M NaCl, with at least five times the column volume to wash the resin to remove the original preservation solution, and then adjust the slurry concentration to 50-70% (v/v) with the packing buffer.

Pack the column(NmXK 16/20 as an example)

- 1) Quickly rinse the connector at the end of the column with the loading solution to remove air bubbles, then close the column outlet, with 1-2 cm of loading solution remaining at the bottom of the column.
- 2) Remove air bubbles from the upper column head pipeline with the loading solution.
- 3) Resuspend the resin, divert the flow with a glass rod against the inner wall of the column, pour the gel suspension continuously into the column, clean the column wall with the loading solution and fill the column tube, and install the upper column head properly. Avoid the introduction of air bubbles during the operation.
- 4) Open the outlet at the bottom of the column, turn on the chromatographic system pump and press the column with constant flow or constant pressure within the recommended pressure range. Mark the gel interface properly when the column bed is stabilized.
- 5) Turn off the pump and close the column outlet, unscrew the upper column inlet pipeline, push the column bed with the upper column head to 2-3 mm below the mark line, and then tighten the upper column inlet pipeline.

Qualify the column

The packed column is equilibrated with 2 CV of 0.5 M NaCl solution and then tested with 2.0 M NaCl at a flow rate of 100 cm/h. The column can also be equilibrated with deionized water and tested with acetone solution. See Table 3 below for specific test parameters.

Table 3. Conditions of column qualification.

Sample	5% (v/v) acetone/water or 2 M NaCl
Plug volume	1 ~ 5 % CV
Mobile phase	Deionized water or 5 M NaCl
Flow rate	50 ~ 200 cm/h
Detection	5 % acetone/water: UV @ 280 nm 2 M NaCl: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m) : >2500

Chromatography method

- 1) Rinsing and equilibration
Rinse the column with elution buffer (e.g. 50 mM sodium citrate, pH 2.5) and equilibrate the column with equilibration buffer (e.g. 20 mM PB + 150 mM NaCl, pH 7.4) before use;
- 2) Sample injection
The sample amount does not exceed 0.8 times the resin DBC_{10%};
- 3) Cleaning
5 CV of equilibrium buffer (e.g. 20 mM PB + 150 mM NaCl, pH 7.4);
- 4) Elution
5 CV of citric acid, pH 2.5;
- 5) Cleaning
5 CV of 1 M acetic acid;
- 6) Regeneration (Cleaning-in-place, CIP)
Clean with 3 - 5 CV of 6 M guanidine hydrochloride, and extend the soaking time if necessary;
- 7) Re-equilibrium
Wash with 5 CV of equilibration buffer (e.g., 20 mM PB + 150 mM NaCl, pH 7.4) to baseline;
- 8) Short-term storage
Replace the buffer with deionized water and 20% ethanol respectively, and store in 20% ethanol.

Long-term storage

Seal and store the column/resin in 20 % ethanol at a recommended storage temperature of 2-8°C. It is recommended to replace it with fresh 20% ethanol every 3 months to prevent ethanol evaporation and microbial growth.

Note: All samples and mobile phases must be filtered with a 0.45 µm filter before use.

Troubleshooting Guide

If you encounter any difficulties while utilizing NMab ProtL, please refer to the table below for potential solutions or feel free to contact us for further assistance.

Observation	Possible cause	Recommended action
Increase in column pressure	Too high flow rate	Reduce the flow rate.
	Clogged on-line filter of instrument	Remove impurities and wash or replace the filter, and filter the sample and buffer prior to use.
	Column bed compressed	Repack the column.
	Long service time of the chromatographic column	Replace the chromatographic column or replace chromatographic medium.
	Valve between pump and collector not opened	Open the valve.
Precipitation observed on the column	Accumulation of hydrophobic proteins, lipoproteins or lipids	Rinse with a nonionic surfactant (e.g., 0.1% Tergitol 15-S-9 or Triton X-100 reduced/reduced), or 0.5 M NaOH, or 1 M guanidine hydrochloride; then rinse with at least 5 times the column volume of sterilized and filtered binding buffer (pH 7-8).
	Protein denaturation and precipitation	Rinse the column with 2 times the column volume of 50 mM NaOH, or 50 mM NaOH and 1.0 M NaCl, or 0.1 M H ₃ PO ₄ , or 6 M guanidine hydrochloride and 10 mM NaOH for at least 10 min; then rinse with at least 5 times the column volume of sterilized and filtered binding buffer (pH 7-8).

Order information

Product name	Pack size	Cat. No.
NMab ProtL	2 mL	17050-070100-2002
	5 mL	17050-070100-2005
	25 mL	17050-070100-2025
	100 mL	17050-070100-2100
	1 L	17050-070100-1001

Note: Prepacked columns of 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm are available. Please contact us for further details.

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