

Ligatrap® Human IgM Purification Column Product Instructions

Introduction

Ligatrap® Technologies now offers our various lines of antibody affinity chromatography resins in a 1 and 5mL prepacked column format for your research and process development needs. Ligatrap® Human IgM Prepacked Columns are capable of binding $\geq 15\text{mg}$ monoclonal Human IgM /ml Resin. Kappa and Lambda IgM can be purified using this product. Ligatrap® IgM purification resin is capable of processing and purifying monoclonal antibodies from cell culture supernatant, ascites fluid, hybridoma, and other sources of recombinant IgM. **Serum applications are not recommended with all Ligatrap® IgM Purification products, due to potential cross reactivity with other immunoglobulins.**



Chromatographic Procedure Outline

All buffers can be prepared as shown in Table 1 below, or can be purchased as pre-qualified buffers from <https://www.ligatrap.com/product/all-products/>

Part #	Name	Formulation
BU-131-FP	Ligatrap® Sample Diluent 2.0	50mg/mL Adipic Acid, 4.0 M NaCl, pH 5.8
BU-132-FP	Ligatrap® Equilibration/Wash Buffer 2.0	10mg/mL Adipic Acid, 800mM NaCl, pH 5.8
BU-133-FP	Ligatrap® IgM Elution Buffer	0.05M Sodium Acetate, pH 3.8
BU-124-FP	Ligatrap® Regeneration Buffer	0.1M Glycine, pH 2.5
BU-125-FP	Ligatrap® Neutralization Buffer	3.0M Tris-Base, pH 11.1
BU-126-FP	Ligatrap® Storage Buffer	10mM Sodium Phosphate, 0.15M NaCl, 0.05% Sodium Azide, pH 7.2

Table 1: Ligatrap Chromatographic Buffers and formulation

Note: Adipic Acid can be purchased from Sigma (A26357-500G)

Note: Adipic Acid is insoluble at low pH. It will solubilize as the pH increases to > 5.0 . For example, after adding all components of Ligatrap Sample Diluent 2.0 Buffer, a 500ml batch will require $\sim 60\text{mL}$ of 5N NaOH to begin dissolving the adipic acid. Continue to titrate with NaOH until a final pH of 5.8 is achieved. Then QS to the desired volume.

Note: For best results, **titrate Ligatrap® IgM Elution Buffer with Glacial Acetic Acid.**

Note: To limit precipitation of Tris-Base, **store neutralization buffer at room temperature.**

Prepare Sample for Column Loading

- ❖ Add *LigaTrap® Sample Diluent 2.0* to the sample containing Human IgM at a ratio of 1:4
(Example: Add 2 mL *LigaTrap® Sample Diluent 2.0* to 8 mL of sample, or 200 mL to 800 mL of sample, etc...)
- ❖ Clarify sample via centrifugation to minimize risk of clogging column with particulate matter.
 - Recommended Speed: 10,000xg for 10-15 minutes.
 - It may be beneficial depending on sample matrix, to pass material through 0.22-0.45µm filter to remove remaining insoluble components.

Connection of Column to Chromatography System

Ensure not to exceed a maximum pressure of 0.2 MPa (2 Bar)

- ❖ To remove cap on outlet side of column, be sure to twist off cap. **DO NOT SNAP OFF**. Incorrect removal of cap can negatively impact column performance.
- ❖ Connect column to system using correct connectors. Make drop-to-drop connection with column using either *LigaTrap® Storage Buffer* or *LigaTrap® Equilibration/Wash Buffer 2.0*.
 - **Recommended flow rate for connection: 0.5-1.0 mL/minute**
 - *If using a FPLC system capable of setting multiple pressure alarms, set the pre-column pressure alarm to 0.2MPa.*

Processing Step	Recommended Flow Rate (mL/minute)
Equilibration	1mL Column: 1.0-2.0 mL/minute 5mL Column: 3.0-5.0 mL/minute
Sample Load/Wash/Elution/Regeneration/Sanitization	1mL Column: 0.1-0.2 mL/minute 5mL Column: 0.5-1.0 mL/minute

Table 2. Recommended Flow Rates

Removal of Storage Buffer and Column Equilibration

- ❖ After making connection to system, begin equilibrating with *LigaTrap® Equilibration/Wash Buffer 2.0*. Equilibrate the column with at least 10 CV (column volumes) to ensure complete removal of storage buffer.

Application of Sample

- ❖ Load prepared sample (as described above) over column. For best results allow for residence time of 5-10 minutes to ensure maximum binding of Human IgM.

Wash

- ❖ Following loading of sample, wash the column with 10-15 CV of *LigaTrap® Equilibration/Wash Buffer 2.0*.

Elute

- ❖ Elute bound antibody with 5-10 CV of *LigaTrap® IgM Elution Buffer*. For higher concentration elute with 5 CV, but if higher yields are desired, use 10 CV.
 - Make sure to keep track of which elution scheme used for future buffer exchange and/or pH adjustment. Add *LigaTrap® Neutralization Buffer* at a volume equal to 12% v/v of total elution volume.

Regeneration

- ❖ Regenerate column with 10 CV of *LigaTrap® Regeneration Buffer*.

Re-Equilibration/Storage

- ❖ If more runs are desired, re-equilibrate column with 10 CV of *LigaTrap® Equilibration/Wash Buffer 2.0*, to prepare column for next run.

- ❖ If column will not be used for an extended period of time, wash column with 10CV of *LigaTrap® Storage Buffer* to remove any residual processing buffers. Cap both ends and store at 2-8° C.

Column Maintenance

After extended use, the column may non-specifically bind small amounts of impurities, leading to a loss in column performance. It is recommended that a 0.5M NaOH solution be used for sanitization of the column.

- ❖ Sanitize the column with 10CV of 0.5M NaOH. **A contact time of 20 minutes is recommended** for sufficient removal of any bound impurities.
 - **DO NOT** leave column in 0.5M NaOH for extended periods of time, as high pH and corrosive nature of NaOH could negatively impact column performance.
 - Use at least 10CV *LigaTrap® Storage Buffer*, to ensure the column is properly neutralized before running chromatographic protocol or storage.

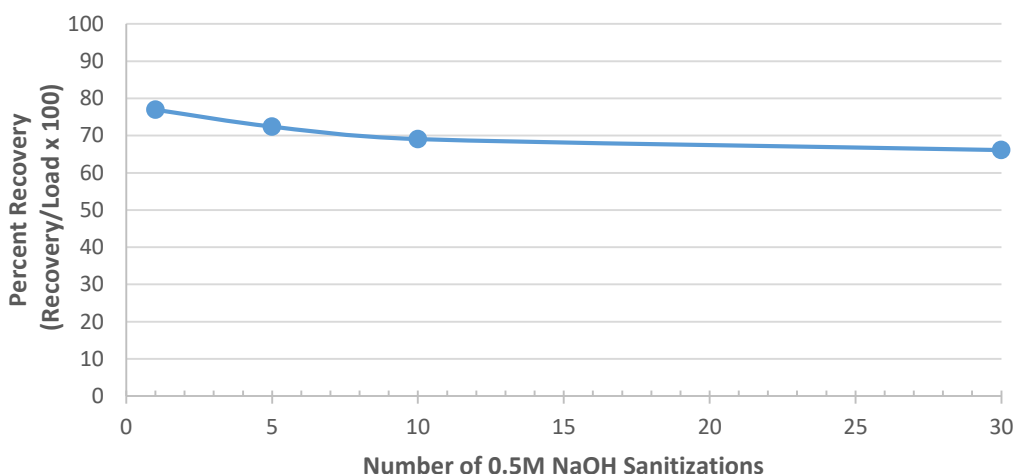


Figure 1: LigaTrap® Prepacked Column alkaline stability. Load: 15 mg IgM /mL Resin. 0.5M NaOH contact time of 20 minutes per cycle.

Product Specifications

Parameter	LigaTrap® Prepacked Column Specification
Ligand Binding Target	Human IgM
Ligand	LigaTrap® Human IgM Affinity Ligand
Binding Capacity	≥15 mg Human IgM /mL Resin
Column Volume	1 or 5 mL
Column Dimensions	7.4 x 25.3 mm (1 mL Column) 15.8 x 26.2 mm (5 mL Column)
Recommend Flow Rates	1 mL Column: 0.1 - 2.0 mL/minute 5mL Column: 1.0 - 5.0 mL/minute
Pressure Limit	0.2 MPa (2.0 Bar)
pH Stability	3-10 Extended Exposure 1-14 Sanitization
Temperature Stability	2 - 42° C Long Term Storage 2-8° C
Storage	2-8° C in 10mM Sodium Phosphate, 0.15M NaCl, 0.05% Sodium Azide, pH 7.2

Other LigaTrap® Products

Target Species	Isotype	Part Number		
		Prepacked Columns	5mL Loose Resin	Purification Spin Column Kit
Human	IgG	LT-095-1x1ml LT-095-3x1ml LT-095-1x5ml	LT-095	LT-095KIT
	IgM	LT-143-1x1ml LT-143-3x1ml LT-143-1x5ml	LT-143	LT-143KIT
	IgA	LT-146-1x1ml LT-146-3x1ml LT-146-1x5ml	LT-146	LT-146KIT
Mouse	IgG	LT-137-1x1ml LT-137-3x1ml LT-137-1x5ml	LT-137	LT-137KIT
	IgM	LT-145-1x1ml LT-145-3x1ml LT-145-1x5ml	LT-145	LT-145KIT
Rat	IgG	LT-138-1x1ml LT-138-3x1ml LT-138-1x5ml	LT-138	LT-138KIT
	IgM	LT-147-1x1ml LT-147-3x1ml LT-147-1x5ml	LT-147	LT-147KIT
Sheep	IgG	LT-141-1x1ml LT-141-3x1ml LT-141-1x5ml	LT-141	LT-141KIT
Llama	IgG	LT-144-1x1ml LT-144-3x1ml LT-144-1x5ml	LT-144	LT-144KIT
Goat	IgG	LT-136-1x1ml LT-136-3x1ml LT-136-1x5ml	LT-136	LT-136KIT
Rabbit	IgG	LT-139-1x1ml LT-139-3x1ml LT-139-1x5ml	LT-139	LT-139KIT
Chicken	IgY	LT-142-1x1ml LT-142-3x1ml LT-142-1x5ml	LT-142	LT-142KIT

For further product information please visit our website at LigaTrap.com. For technical support and questions email us at techsupport@ligatrap.com