

LigaTrap® Llama IgG Purification Column Product Instructions

Introduction

LigaTrap® Technologies now offers our various lines of antibody affinity chromatography resins in a 1 and 5 mL prepacked column format for your research and process development needs. LigaTrap has developed a novel, patented, series of affinity ligands specific for the purification of monoclonal and polyclonal immunoglobulins from various species. LigaTrap® Prepacked Columns are capable of processing and purifying antibodies from several matrices, including cell culture supernatant, ascites fluid, and serum.



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/purification-buffers/>

Part #	Name	Composition
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-123-FP	LigaTrap® Elution Buffer	0.1M Sodium Acetate, pH 4.0
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 Composition

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 ~60mL 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® 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 **llama IgG** 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 0.5 MPa (5 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**

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 **llama IgG**.

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® 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.

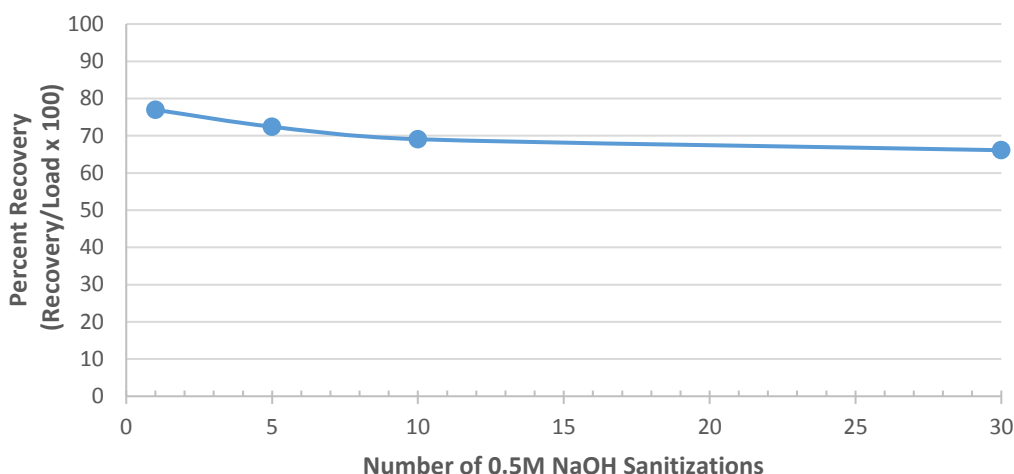


Figure 1: LigaTrap® Prepacked Column alkaline stability. Load: 15 mg IgG/mL Resin. 0.5M NaOH contact time of 20 minutes per cycle.

Product Specifications

Parameter	LigaTrap® Prepacked Column Specification
Ligand Binding Target	Llama IgG
Ligand	LigaTrap® Llama IgG Affinity Ligand
Binding Capacity	≥15 mg Llama IgG/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	0.5 MPa (5.0 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