LigaTrap® Llama IgG 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 Llama IgG Prepacked Columns are capable of binding ≥ 15 mg monoclonal Llama IgG /mL Resin. Kappa and Lambda IgG may be purified using this product. LigaTrap Llama IgG Purification Resin is capable of processing and purifying monoclonal antibodies form cell culture supernatant, ascites fluid, hybridoma, and other sources of recombinant IgG.

Chromatographic Procedure Outline

All buffers can be prepared as shown in **Table 1** below, or can be purchased as pre-qualified buffers from the LigaTrap Technologies website.

Table 1: LigaTrap Chromatographic Buffers and Composition

Part #	Name	Composition
BU-131-FP	**LigaTrap Sample Diluent 2.0**	50mg/mL Adipic Acid, 4.0M 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

Note: **Adipic Acid is insoluble at low pH**. It will solubilize as the pH increases to > 5.0.
Note: For best results, **titrate LigaTrap Elution Buffer with Glacial Acetic Acid.**
Note: To limit precipitation of Tris-Base, **store LigaTrap Neutralization Buffer at room temperature**.
Note: **Equilibrate all buffers to room temperature prior to use**.

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 0.22-0.45um 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.***

Table 2. Recommended Flow Rates

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

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 15-18% v/v of total elution volume.

Regeneration

- Regenerate column with 5-10 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 10 CV 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 10 CV 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 10 CV *LigaTrap Storage Buffer*, to ensure the column is properly neutralized before running chromatographic protocol or storage.

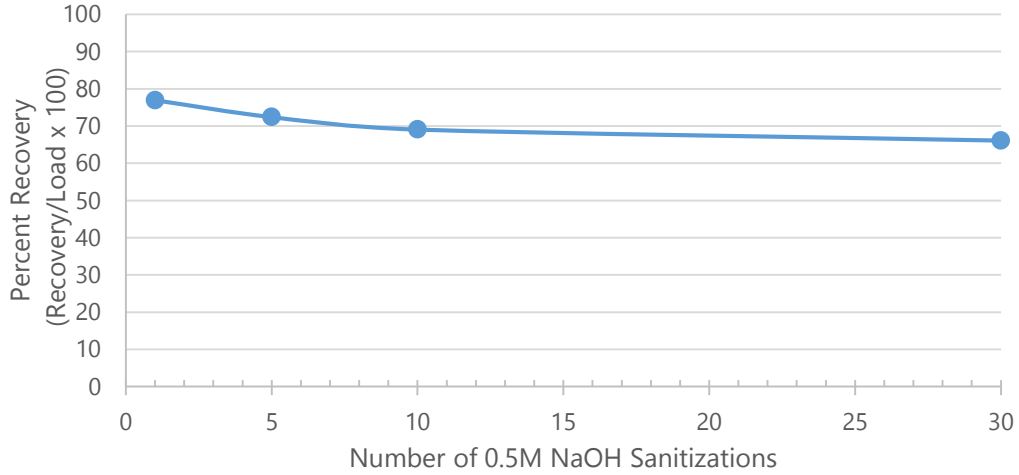

Figure 1: LigaTrap Prepacked Column alkaline stability. Load: 15 mg IgG /mL Resin. 0.5M NaOH contact time of 20 minutes per cycle.

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	0.2 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:

		Part Number			
Target Species	**Antibody**	**Loose Resin**	**Microspin Columns**	**Prepacked Columns**	**Purification Kits**
Human	IgG	LT-095	LT-095-MSC	LT-095-1x1mL LT-095-3x1mL LT-095-1x5mL	LT-095KIT LT-095-1mL KIT LT-095-5mL KIT
	IgM	LT-143	LT-143-MSC	LT-143-1x1mL LT-143-3x1mL LT-143-1x5mL	LT-143KIT LT-143-1mL KIT LT-143-5mL KIT
	IgA	LT-146	LT-146-MSC	LT-146-1x1mL LT-146-3x1mL LT-146-1x5mL	LT-146KIT LT-146-1mL KIT LT-146-5mL KIT
Mouse	IgG	LT-137	LT-137-MSC	LT-137-1x1mL LT-137-3x1mL LT-137-1x5mL	LT-137KIT LT-137-1mL KIT LT-137-5mL KIT
	IgM	LT-145	LT-145-MSC	LT-145-1x1mL LT-145-3x1mL LT-145-1x5mL	LT-145KIT LT-145-1mL KIT LT-145-5mL KIT
Rat	IgG	LT-138	LT-138-MSC	LT-138-1x1mL LT-138-3x1mL LT-138-1x5mL	LT-138KIT LT-138-1mL KIT LT-138-5mL KIT
	IgM	LT-147	LT-147-MSC	LT-147-1x1mL LT-147-3x1mL LT-147-1x5mL	LT-147KIT LT-147-1mL KIT LT-147-5mL KIT
Llama	IgG	LT-144	LT-144-MSC	LT-144-1x1mL LT-144-3x1mL LT-144-1x5mL	LT-144KIT LT-144-1mL KIT LT-144-5mL KIT
Goat	IgG	LT-136	LT-136-MSC	LT-136-1x1mL LT-136-3x1mL LT-136-1x5mL	LT-136KIT LT-136-1mL KIT LT-136-5mL KIT
Rabbit	IgG	LT-139	LT-139-MSC	LT-139-1x1mL LT-139-3x1mL LT-139-1x5mL	LT-139KIT LT-139-1mL KIT LT-139-5mL KIT
Chicken	IgY	LT-142	LT-142-MSC	LT-142-1x1mL LT-142-3x1mL LT-142-1x5mL	LT-142KIT LT-142-1mL KIT LT-142-5mL KIT

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