

# LigaTrap® Rat 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	1.0M Tris-Base, pH 8.5
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

## **Prepare Sample for Column Loading**

- ❖ Add LigaTrap® Sample Diluent 2.0 to the sample containing **rat 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 0.5 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: 5.0-7.0 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 **rat 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% of total elution volume.

## Regeneration

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

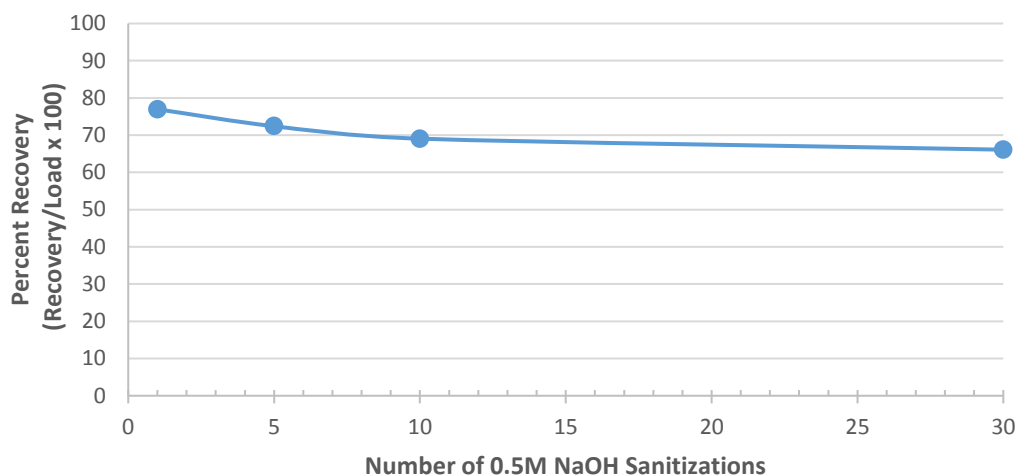
## Re-Equilibration/Storage

- ❖ 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: 20 mg IgG/mL Resin. 0.5M NaOH contact time of 20 minutes per cycle.

## Product Specifications

Parameter	LigaTrap® Prepacked Column Specification
<b>Ligand Binding Target</b>	Rat IgG
<b>Ligand</b>	LigaTrap® Rat IgG Affinity Ligand
<b>Binding Capacity</b>	> 20 mg Rat IgG/mL Resin
<b>Column Volume</b>	1 or 5 mL
<b>Column Dimensions</b>	7.4 x 25.3 mm (1 mL Column) 15.8 x 26.2 mm (5 mL Column)
<b>Recommend Flow Rates</b>	1 mL Column: 0.1 - 2.0 mL/minute 5mL Column: 1.0 - 5.0 mL/minute
<b>Maximum Flow Rates</b>	1mL Column: 10.0 mL/minute 5 mL Column: 15.0 mL/minute
<b>Pressure Limit</b>	0.5 MPa (5.0 Bar)
<b>pH Stability</b>	3-10 Extended Exposure 1-14 Sanitization
<b>Temperature Stability</b>	4 - 42° C Long Term Storage 2-8° C
<b>Storage</b>	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
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](http://LigaTrap.com). For technical support and questions email us at [techsupport@ligatrap.com](mailto:techsupport@ligatrap.com)