

# LigaTrap® Human IgM Loose Resin Protocol

## Introduction

LigaTrap® Technologies has developed a novel, patented, series of affinity ligands specific for the purification of monoclonal and polyclonal immunoglobulins from various species. **LigaTrap® Human IgM Purification resin is capable of binding  $\geq 15\text{mg}$  monoclonal human IgM/ml resin.**

## Chromatographic Buffers:

Part #	Name	Composition
<b>BU-131-FP</b>	<b>LigaTrap Sample Diluent 2.0</b>	50mg/ml Adipic Acid, 4.0M NaCl, pH 5.8
<b>BU-132-FP</b>	<b>LigaTrap Equilibration/Wash Buffer 2.0</b>	10mg/ml Adipic Acid, 800mM NaCl, pH 5.8
<b>BU-123-FP</b>	<b>LigaTrap Elution Buffer</b>	0.1M Sodium Acetate, pH 4.0
<b>BU-124-FP</b>	<b>LigaTrap Regeneration Buffer</b>	0.1M Glycine, pH 2.5
<b>BU-125-FP</b>	<b>LigaTrap Neutralization Buffer</b>	3.0M Tris-Base, pH 11.1
<b>BU-126-FP</b>	<b>LigaTrap Storage Buffer</b>	10mM Sodium Phosphate, 0.15M NaCl, 0.05% Sodium Azide, pH 7.2

**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® Elution Buffer with Glacial Acetic Acid

**Note:** To limit precipitation of Tris-Base, store LigaTrap Neutralization Buffer at room temperature.

## Prepare Sample For Binding:

1. Add the **LigaTrap Sample Diluent 2.0** to the sample containing IgM at a ratio of 1:4 (Example: Add 2ml LT Sample Diluent 2.0 to 8ml of sample, or 200ml to 800ml of sample, etc...)

## Chromatographic Protocol:

**Pressure Max = 2 Bar/0.2MPa**

- Pack the appropriately sized column volume (CV), for your particular application.
  - Recommend 10 minute residence time for the sample load portion of the purification cycle
  - Recommend 5 minute residence time for all other chromatographic steps
1. Equilibrate the resin with 10-20 CV of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**
  2. Load the "prepared" sample (described above)
  3. Wash the resin with 15CV (if Phenol Red is present in sample wash with 30-35CV to completely wash out color) of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**
  4. Elute bound antibody with 10 CV of **LigaTrap Elution Buffer (BU-123-FP)**. Note, for higher concentration antibody elute with 5 CV, but if higher yields are desired, use 10 CV. Neutralize with 10-12% v/v of **LigaTrap Neutralization Buffer**.
  5. Regenerate the column with 10 CV of **LigaTrap Regeneration Buffer (BU-124-FP)**
  6. Re-Equilibrate the resin with 10 CV **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)** if an additional purification cycle will be done.

7. If purification is complete, store the resin by running 5 CV of **LigaTrap Storage Buffer (BU-126-FP)** over the column. Or if sanitization is desired, run 0.5M Sodium Hydroxide (NaOH) over resin with a contact time of 20-30 minutes followed by 10CV of **LigaTrap Storage Buffer (BU-126-FP)**.

## **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](http://LigaTrap.com). For technical support and questions email us at [techsupport@ligatrap.com](mailto:techsupport@ligatrap.com)