

# LigaTrap<sup>®</sup> Human IgM Loose Resin

## Product Instructions

### Introduction:

LigaTrap Human IgM Purification Resin is capable of binding  $\geq 15$  mg monoclonal Human IgM /mL Resin. Kappa and Lambda IgM may be purified using this product. LigaTrap Human 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 Buffers:

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-133-FP	LigaTrap IgM Elution Buffer	500mM 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

**Note:** Adipic Acid is insoluble at low pH. It will solubilize as the pH increases to  $> 5.0$ .

**Note:** For best results, titrate LigaTrap IgM 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 Binding:

1. Add the **LigaTrap Sample Diluent 2.0 (BU-131-FP)** to the sample containing Human IgM at a ratio of 1:4. (For example: Add 2 mL LigaTrap Sample Diluent 2.0 to 8mL of sample, or 200mL to 800mL of sample, etc...)

### Chromatographic Protocol:

- Pack the appropriately sized column volume (CV), for your particular application. **Ensure not to exceed a maximum flow rate of 600cm/hr.**
  - If using a FPLC system capable of setting multiple pressure alarms, set the pre-column pressure alarm to 0.2 MPa.
  - Recommended 5-10 minute residence times for the sample load and all other chromatographic steps
1. Equilibrate the resin with 10 CV of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**.
  2. Load the prepared sample (described above).
  3. Wash the resin with 10-15 CV of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**.
  4. Elute bound antibody with 10 CV of **LigaTrap IgM Elution Buffer (BU-133-FP)**.
  5. Neutralize with 12.5% v/v of **LigaTrap Neutralization Buffer (BU-125-FP)**.
  6. Regenerate the column with 10 CV of **LigaTrap Regeneration Buffer (BU-124-FP)**.
  7. Re-Equilibrate the resin with 10 CV **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)** if an additional purification cycle will be done.
  8. If purification is complete, store the resin by running 5 CV of **LigaTrap Storage Buffer (BU-126-FP)** over the column and store at 2-8 °C.

### **Batch Method Preparation (Optional):**

1. Transfer loose resin to a clean mixing container of adequate volume to hold resin volume plus sample volume and buffers.
2. Add mixture of sample with **LigaTrap Sample Diluent 2.0 (BU-131-FP)** into this container and mix gently for desired time, 1-2 hours mixing is recommended for a sample with very low immunoglobulin concentration or overnight at 4 °C.
3. Remove the supernatant after incubation and wash the resin with 5-10 CV of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**, collect these washes.
4. Add **LigaTrap IgM Elution Buffer (BU-133-FP)** and incubate for 5-10 minutes, collect the eluates.  
**Note:** This collected elution buffer has immunoglobulin, do not discard.
5. Neutralize eluates with 12.5% v/v **LigaTrap Neutralization Buffer (BU-125-FP)** and gently vortex.
6. Regenerate the resin with 5-10 CV of **LigaTrap Regeneration Buffer (BU-124-FP)** with 5-10 minutes mixing and collect the flow through.
7. If the resin will be reused, re-equilibrate with 10 CV **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**. Discard these washes.
8. If purification is complete, store the resin by adding 1 CV of **LigaTrap Storage Buffer (BU-126-FP)** into the resin and store at 2-8 °C.

**Other LigaTrap Products:**

Target Species	Antibody	Part Number			
		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](http://LigaTrap.com). For technical support and questions, email us at [info@ligatrap.com](mailto:info@ligatrap.com)