

LigaTrap[®] Rat IgM Loose Resin Product Instructions

Introduction:

LigaTrap Rat IgM Purification Resin is <u>capable of binding \geq 10 mg monoclonal Rat IgM /mL Resin</u>. Kappa and Lambda IgM may be purified using this product. LigaTrap Rat IgM Purification Resin is capable of processing and purifying monoclonal antibodies form 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		

<u>Note</u>: Adipic Acid is insoluble at low pH. It will solubilize as the pH increases to > 5.0. <u>Note</u>: 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.

Prepare Sample for Binding:

1. Add the **LigaTrap Sample Diluent 2.0 (BU-131-FP)** to the sample containing Rat 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 pressure of 0.2 MPa (2 Bar).
- 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.
- 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.
- 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.
- 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:

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