

LigaTrap® Human IgM Loose Resin Protocol

Introduction

LigaTrap® Human IgM Prepacked Columns are capable of binding ≥ 15 mg monoclonal Human IgM /ml Resin. Kappa and Lambda IgM can be purified using this product. LigaTrap® 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	0.05M 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 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 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 IgM at a ratio of 1:4 (For example: Add 2ml LigaTrap Sample Diluent 2.0 to 8ml of sample, or 200ml to 800ml of sample, etc...)

Batch Method Preparation (Optional)

- Pack the appropriately sized column volume (CV), for your particular application. **Ensure not to exceed a maximum pressure of 0.2 MPa (2 Bar).**
- Recommend 5-minute residence times for the Sample Load and all other chromatographic steps
- *If using a FPLC system capable of setting multiple pressure alarms, set the pre-column pressure alarm to 0.2MPa.*
 1. Equilibrate the resin with 10CV of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**.
 2. Transfer loose resin from column to clean mixing container of adequate volume to hold resin volume plus sample volume with excess room
 3. Allow sample and resin to mix with gentle stirring for 24 hours, at 4 °C if possible
 4. Return resin and sample mixture to closed empty column for repacking, allowing resin bed to settle
 5. Allow supernatant to run over resin bed until sample meniscus is approximately 1-5 mm above top of column bed. Collect this as the Flow Through fraction.
 6. Continue from number 3 in "**Chromatographic Protocol**"

Chromatographic Protocol

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➤ If using a FPLC system capable of setting multiple pressure alarms, set the pre-column pressure alarm to 0.2MPa.

1. Equilibrate the resin with 10CV of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**
2. Load the "prepared" sample (described above)
3. Wash the resin with 10-15CV 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)**. Note, for higher concentration antibody elute with 5 CV, but if higher yields are desired, use 10 CV. Neutralize with 12% v/v of **LigaTrap Neutralization Buffer (BU-125-FP)**.
5. Regenerate the column with 10CV of **LigaTrap Regeneration Buffer (BU-124-FP)**
6. Re-Equilibrate the resin with 10CV **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 5CV of **LigaTrap Storage Buffer (BU-126-FP)** over the column.

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-138-1"x1ml 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. For technical support and questions email us at techsupport@ligatrap.com