

# LigaTrap<sup>®</sup> Human IgM Microspin Columns

## Product Instructions

### Introduction

LigaTrap Human IgM Purification Resin is engineered to purify high quality Human IgM antibodies from polyclonal and monoclonal sources. LigaTrap Human IgM Purification Resin is capable of binding  $\geq 15$  mg Human IgM /mL resin. Each microspin column may be used, regenerated, and reused up to 10 times with minimal loss in binding capacity. Kappa and Lambda IgM may be purified using this product. **Serum applications are not recommended with all LigaTrap IgM Purification products, due to potential cross reactivity with other immunoglobulins.**

**Table 1.** Product Contents

Part #	Item	Quantity
LT-143-MSC	<b>Microspin Columns-</b> centrifuge columns supplied with caps and plug. Each microspin column contains 0.1 mL LigaTrap Human IgM Purification Resin in LigaTrap Storage Buffer.	<b>10</b>

### Additional Materials Required

#### Buffers

All buffers can be prepared as shown in **Table 2** below, or can be purchased as pre-qualified buffers from the LigaTrap Technologies website.

**Table 2.** Chromatographic Buffers

Part #	Name	Composition
BU-131-FP	<b>LigaTrap Sample Diluent 2.0</b>	50mg/mL Adipic Acid, 4.0M NaCl, pH 5.8
BU-132-FP	<b>LigaTrap Equilibration/Wash Buffer 2.0</b>	10mg/mL Adipic Acid, 800mM NaCl, pH 5.8
BU-133-FP	<b>LigaTrap IgM Elution Buffer</b>	500mM Sodium Acetate, pH 3.8
BU-124-FP	<b>LigaTrap Regeneration Buffer</b>	0.1M Glycine, pH 2.5
BU-125-FP	<b>LigaTrap Neutralization Buffer</b>	3.0M Tris-Base, pH 11.1
BU-126-FP	<b>LigaTrap Storage Buffer</b>	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.**

### Equipment

- Microcentrifuge set between 1000-3000 x g
- Vortex/Mixer
- Centrifugation tubes or container for sample preparations

## Antibody Purification Procedure

### Sample Preparation

1. In a separate tube add 320µL of sample matrix (i.e. hybridoma supernatant or cell culture fluid) containing Human IgM.
2. Add 80µL of **LigaTrap Sample Diluent 2.0 (BU-131-FP)** to the sample. Mix briefly by vortexing.

### Purification

3. Snap off the bottom plug on the microspin column. **Save this plug, as it will be needed to stopper the column.**
4. Insert the microspin column into a 2.0 mL collection tube. Equilibrate resin by adding 400µL of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)** to the unplugged microspin column. Centrifuge between 1000-3000 x g for 1 minute. Discard the buffer in collection tube. Repeat for two additional 400µL equilibrations. Insert the bottom plug onto the microspin column.
5. Transfer 400µL of the prepared sample (from Step # 2) to the equilibrated column. Place screw cap on snugly. Continue to mix/shake the sample and resin continuously for **5 minutes**. Remove bottom plug and insert microspin column into a new 2.0 mL collection tube. Centrifuge between 1000-3000 x g for 1 minute. Retain flow through.
6. Insert bottom plug onto the microspin column and add 400µL of the **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**. Mix/shake resin continuously for 5 minutes. Remove bottom plug and insert microspin column into a new 2.0 mL collection tube. Centrifuge between 1000-3000 x g for 1 minute. Retain wash flow through. Repeat process for a second 400µL wash.
7. Insert the bottom plug onto the microspin column and add 400µL of **LigaTrap IgM Elution Buffer (BU-133-FP)**. Mix/shake resin continuously for 5 minutes. Remove bottom plug and insert the microspin column into a new 2.0 mL collection tube labeled **Eluate 1**. Centrifuge between 1000-3000 x g for 1 minute. Repeat process for a second 400µL elution and use a new 2.0 mL collection tube labeled **Eluate 2**.  
**Note:** The eluates contain the purified antibodies. **Do not discard!**
8. Add 50µL (12.5% v/v of elution samples) of **LigaTrap Neutralization Buffer (BU-125-FP)** to each of the eluates obtained in Step # 7. Vortex briefly. The antibody will be near neutral pH and is ready for downstream applications.  
**Note:** There are no preservatives in the antibody. Use the antibody within one week or aliquot and store at -20° C or colder. Avoid multiple freeze thaws.
9. Insert the bottom plug onto the microspin column and add 400µL of **LigaTrap Regeneration Buffer (BU-124-FP)**. Mix/shake resin continuously for 5 minutes. Remove bottom plug and insert microspin column into a new 2.0 mL collection tube. Centrifuge between 1000-3000 x g for 1 minute. Retain regeneration flow through.
10. Add 50µL of **LigaTrap Neutralization Buffer (BU-125-FP)** to the regeneration flow through obtained in Step # 9. Vortex briefly.
11. If the column will not be reused, it may be discarded. If the microspin column is to be reused, re-equilibrate the resin by repeating the process described in Step # 4.

12. To store resin, remove bottom plug and insert microspin column into a new 2.0 mL collection tube. Add 400µL of **LigaTrap Storage Buffer (BU-126-FP)**. Centrifuge between 1000-3000 x g for 1 minute. Repeat for two more 400µL washes. Once complete, insert the bottom plug onto the microspin column and add 400µL of fresh **LigaTrap Storage Buffer (BU-126-FP)**. Store plugged microspin column upright 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 [www.LigaTrap.com](http://www.LigaTrap.com). For technical support and questions, email us at [info@ligatrap.com](mailto:info@ligatrap.com)