LigaTrap® IgM Microspin Columns
Product Instructions

Introduction

LigaTrap IgM Purification Resin is engineered to purify high quality IgM antibodies from polyclonal and monoclonal sources. LigaTrap IgM Purification Resin is recommended for Human, Mouse, or Rat IgM applications. 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-155-MSC	**Microspin Columns-** centrifuge columns supplied with caps and plug. Each microspin column contains 0.1 mL LigaTrap IgM Purification Resin in LigaTrap Storage Buffer.	**10**

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	**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.**

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 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:

		Part Number			
Product	**Antibody**	**Loose Resin**	**Microspin Columns**	**Prepacked Columns**	**Purification Kits**
LigaTrap Base Resin	IgG, IgA, IgY	LT-150	LT-150-MSC	LT-150-1x1mL LT-150-3x1mL LT-150-1x5mL	LT-150KIT LT-150-1mL KIT LT-150-5mL KIT
LigaTrap IgM Resin	IgM	LT-155	LT-155-MSC	LT-155-1x1mL LT-155-3x1mL LT-155-1x5mL	LT-155KIT LT-155-1mL KIT LT-155-5mL KIT

For further product information, please visit our website at www.LigaTrap.com. For technical support and questions, email us at info@ligatrap.com