

# LigaTrap<sup>®</sup> Mouse IgG Microspin Columns

## Product Instructions

### Introduction

LigaTrap Mouse IgG Purification Resin is engineered to purify high quality Mouse IgG antibodies from polyclonal and monoclonal sources. LigaTrap Mouse IgG Purification Resin is capable of binding  $\geq 20$  mg Mouse IgG /mL resin. Each microspin column may be used, regenerated, and reused up to 10 times with minimal loss in binding capacity. Kappa and Lambda IgG may be purified using this product.

**Table 1.** Product Contents

| Part #     | Item   | Quantity  |
|------------|--|-----------|
| LT-137-MSC | <b>Microspin Columns-</b> centrifuge columns supplied with caps and plug. Each microspin column contains 0.1 mL LigaTrap Mouse IgG Purification Resin in PBS buffer with 0.05% sodium azide. | <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-123-FP | <b>LigaTrap Elution Buffer</b>                | 0.1M Sodium Acetate, pH 4.0                                   |
| 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 Elution Buffer with Glacial Acetic Acid

**Note:** To limit precipitation of Tris-Base, store LigaTrap Neutralization Buffer at room temperature.

### 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 Mouse IgG.
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 Elution Buffer (BU-123-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<br>LT-095-3x1mL<br>LT-095-1x5mL | LT-095KIT<br>LT-095-1mL KIT<br>LT-095-5mL KIT |
|                | IgM      | LT-143      | LT-143-MSC        | LT-143-1x1mL<br>LT-143-3x1mL<br>LT-143-1x5mL | LT-143KIT<br>LT-143-1mL KIT<br>LT-143-5mL KIT |
|                | IgA      | LT-146      | LT-146-MSC        | LT-146-1x1mL<br>LT-146-3x1mL<br>LT-146-1x5mL | LT-146KIT<br>LT-146-1mL KIT<br>LT-146-5mL KIT |
| Mouse          | IgG      | LT-137      | LT-137-MSC        | LT-137-1x1mL<br>LT-137-3x1mL<br>LT-137-1x5mL | LT-137KIT<br>LT-137-1mL KIT<br>LT-137-5mL KIT |
|                | IgM      | LT-145      | LT-145-MSC        | LT-145-1x1mL<br>LT-145-3x1mL<br>LT-145-1x5mL | LT-145KIT<br>LT-145-1mL KIT<br>LT-145-5mL KIT |
| Rat            | IgG      | LT-138      | LT-138-MSC        | LT-138-1x1mL<br>LT-138-3x1mL<br>LT-138-1x5mL | LT-138KIT<br>LT-138-1mL KIT<br>LT-138-5mL KIT |
|                | IgM      | LT-147      | LT-147-MSC        | LT-147-1x1mL<br>LT-147-3x1mL<br>LT-147-1x5mL | LT-147KIT<br>LT-147-1mL KIT<br>LT-147-5mL KIT |
| Llama          | IgG      | LT-144      | LT-144-MSC        | LT-144-1x1mL<br>LT-144-3x1mL<br>LT-144-1x5mL | LT-144KIT<br>LT-144-1mL KIT<br>LT-144-5mL KIT |
| Goat           | IgG      | LT-136      | LT-136-MSC        | LT-136-1x1mL<br>LT-136-3x1mL<br>LT-136-1x5mL | LT-136KIT<br>LT-136-1mL KIT<br>LT-136-5mL KIT |
| Rabbit         | IgG      | LT-139      | LT-139-MSC        | LT-139-1x1mL<br>LT-139-3x1mL<br>LT-139-1x5mL | LT-139KIT<br>LT-139-1mL KIT<br>LT-139-5mL KIT |
| Chicken        | IgY      | LT-142      | LT-142-MSC        | LT-142-1x1mL<br>LT-142-3x1mL<br>LT-142-1x5mL | LT-142KIT<br>LT-142-1mL KIT<br>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)