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# LigaTrap® Human IgM Purification Kit

## 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. The LigaTrap Human IgM Purification Kit provides all the necessary reagents for fast, convenient micro-scale purification of Human IgM in just 12 easy steps. Each kit contains 10 microspin columns prefilled with 0.1 mL of LigaTrap Human IgM Purification Resin, buffers, and collection tubes. 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.**

### Kit 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 PBS buffer with 0.05% sodium azide. | 10       |
| BU-131-FP  | <b>LigaTrap Sample Diluent 2.0</b>   | 15 mL    |
| BU-132-FP  | <b>LigaTrap Equilibration/Wash Buffer 2.0</b>  | 250 mL   |
| BU-133-FP  | <b>LigaTrap IgM Elution Buffer</b>   | 125 mL   |
| BU-124-FP  | <b>LigaTrap Regeneration Buffer</b>  | 50 mL    |
| BU-125-FP  | <b>LigaTrap Neutralization Buffer</b>  | 15 mL    |
| BU-126-FP  | <b>LigaTrap Storage Buffer</b>   | 50 mL    |
| PL-057     | <b>2.0 mL Collection Tubes</b>   | 80       |

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### Additional Materials Required

- Microcentrifuge set between 1000-3000 x g
- Vortex/Mixer
- Centrifuge 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 supplied 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<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)