

# LigaTrap® Human IgG Purification Kit

## Product Instructions

### Introduction

LigaTrap® Human IgG Purification Resin is engineered to purify high quality Human IgG antibodies from polyclonal and monoclonal sources. Binding capacity for Human IgG is >30 mg/ml resin. The LigaTrap® Human IgG Purification Kit provides all the necessary reagents for fast, convenient micro-scale purification of Human IgG in just 10 easy steps. Each kit contains 10 microspin columns prefilled with 0.1ml of LigaTrap® Human IgG Purification Resin, buffers, and collection tubes. Each spin column can be used, regenerated, and used up to 10 times with minimal loss in binding capacity. Kappa and Lambda IgG can be purified using this product.

### Kit Contents

Part #	Item	Quantity
LT-095-MSC	<b>Microspin Columns-</b> centrifuge columns supplied with caps and plug. Each column contains 0.1 mL LigaTrap® Human IgG 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-123-FP	<b>LigaTrap 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 mL Collection Tubes</b>	80

### Additional Materials Required

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

## Antibody Purification Procedure

### Sample Prep

1. In a separate tube (not supplied in kit) add 320 $\mu$ l of sample matrix (i.e. hybridoma supernatant or cell culture fluid) containing Human IgG.
2. Add 80 $\mu$ l of **LigaTrap Sample Diluent 2.0 (BU-131-FP)** to the sample. Mix briefly by vortexing.

### Purification

3. Equilibrate resin by adding 400 $\mu$ l of **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**. Snap the bottom plug on the microspin column. Save the plug, as it will be needed to stopper the column. Insert the Microspin column into a supplied 2.0 mL Collection Tube. Centrifuge between 1000-3000 x g for 1 minute. Empty the buffer from Collection Tube. Repeat with two additional 400 $\mu$ l equilibrations. Insert the bottom plug into the microspin column.
4. Transfer 400 $\mu$ l of prepared sample (Step # 2) to the equilibrated column. Place screw cap on snugly. Vortex briefly for 15 seconds. Continue to mix/shake the sample and resin continuously for **5 minutes**.
5. Remove plug and insert the microspin column into a Collection Tube. Centrifuge between 1000-3000 x g for 1 minute. Discard unbound material or retain for further evaluation.
6. Add 400 $\mu$ l of the **LigaTrap Equilibration/Wash Buffer 2.0 (BU-132-FP)**. Remove plug and insert microspin column into a clean collection tube. Mix/shake resin continuously for 5 minutes. Centrifuge between 1000-3000 x g for 1 minute. Discard wash. Repeat with three additional washes for a total of four washes. (4x400 $\mu$ l)
7. Insert the washed microspin column into a **new, labeled** collection tube. Add 400 $\mu$ l of **LigaTrap Elution Buffer (BU-123-FP)** to the column. Vortex briefly for 15 seconds. Mix/shake resin continuously for 5 minutes. Centrifuge between 1000-3000 x g for 1 minute. Place the microspin column into a new, labeled Collection Tube. Repeat with second 400 $\mu$ l elution. **\*Note: The eluates contain the purified antibodies. Do not discard!**
8. Pool eluates from Step 7 and add 80 $\mu$ l (10-12% v/v of elution samples) of **LigaTrap Neutralization Buffer (BU-125-FP)** to the antibody. Attach the cap. Vortex briefly. The antibody will be near neutral pH. The antibody 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. If the column will not be reused, it can be discarded. If column is to be reused, regenerate the column by adding 400 $\mu$ l of **LigaTrap Regeneration Buffer (BU-124-FP)**. Vortex briefly for 15 seconds. Mix/shake resin continuously for 5 minutes. Insert the Microspin column into a 2.0 mL collection tube and centrifuge between 1000-3000 x g for 1 minute.
10. To store resin add 400 $\mu$ l of **LigaTrap Storage Buffer (BU-126-FP)**. Insert the Microspin column into a 2.0 mL collection tube. Centrifuge between 1000-3000 x g for 1 minute. Discard wash. Repeat with an additional two washes for a total of three washes. (3x400 $\mu$ l) Insert the bottom plug into the Microspin column, add 400 $\mu$ l of fresh **LigaTrap Storage Buffer (BU-126-FP)** and store at 2-8° C.