



Version 2.0

# LigaTrap™ Rabbit IgG Purification Kit, 10 x 0.15 mL

### **Part** # *LT-139KIT*

### **Kit Contents**

Part #	Item	Quantity
LT-139-SC	<b>Microspin Columns-</b> centrifuge columns supplied with caps and plug. Each column contains 0.15 mL LigaTrap™ Rabbit Resin in PBS buffer with 0.05% sodium azide.	10
BU-131-FP	LT Sample Diluent 2.0	15 mL
BU-132-FP	LT Equilibration/Wash Buffer 2.0	250 mL
BU-123-FP	LT Elution Buffer	125 mL
BU-124-FP	LT Regeneration Buffer	50 mL
BU-125-FP	LT Neutralization Buffer	15 mL
BU-126-FP	LT Storage Buffer	50 mL
PL-057	2 mL Collection Tubes	80

#### Introduction

ImmunoReagents is now offering LigaTrap<sup>TM</sup> purification resins and kits, engineered for antibody purification. LigaTrap<sup>TM</sup> Technologies has developed a novel, patented, series of affinity ligands specific for the purification of monoclonal and polyclonal immunoglobulins from various species. In addition to species-specific ligands, advantages include the ability to elute antibodies at a higher pH (pH 4.0) than protein A and G (pH  $\leq$  3.0), thus reducing the potential for precipitation and inactivation of pH sensitive antibodies.

**LigTrap<sup>™</sup> Rabbit IgG Resin** is engineered to purify high quality rabbit IgG antibodies from recombinant, monoclonal, and polyclonal sources. Binding capacity for rabbit IgG is >20mg/ml resin. The **LigTrap<sup>™</sup> Rabbit IgG Purification Kit** provides all the necessary reagents for fast, convenient micro-scale purification of rabbit IgG in just 10 easy steps. Each kit contains 10 microspin columns prefilled with 0.15ml of **LigTrap<sup>™</sup> Rabbit IgG Resin**, buffers, and collection tubes. Each spin column can be used, regenerated, and used up to 10 times with minimal loss in binding capacity.

#### Additional Materials Required

- Microcentrifuge set between 2000-6000 x g
- Vortex/Mixer
- Centrifuge tubes or container for sample preparations

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# **Antibody Purification Procedure**

## Sample Prep

- In a separate tube (not supplied in kit) add 400μl of sample matrix (i.e. serum or cell culture media) containing rabbit IgG
- 2. Add 100µl of LT Sample Diluent 2.0 to the sample. Mix briefly by vortexing.

#### **Purification**

- 3. Equilibrate resin by adding 500µl of **LT Equilibration/Wash Buffer 2.0.** 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 2000-6000 x g for 1 minute. Empty the buffer from Collection Tube. Repeat with an additional 500µl equilibration. Insert the bottom plug into the Microspin column.
- 4. Transfer 500μl 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 ~10 min.
- 5. Remove plug and insert the Microspin column into a Collection Tube. Centrifuge between 2000-6000 x g for 1 minute. Discard unbound material or retain for further evaluation.
- 6. Add 500μl of the **LT Equilibration/Wash Buffer 2.0**. Remove plug and insert Microspin column into a clean Collection Tube. Centrifuge between 2000-6000 x g for 1 minute. Discard wash. Repeat with two additional washes for a total of three washes. (3x500μl)
- \*IR-Tip: Collection Tubes used to collect washes can be reused.
- 7. Insert the washed Microspin column into a <u>new, labeled</u> Collection Tube. Add 500µl of **LT Elution Buffer** to the column. Vortex briefly for 15 seconds. Centrifuge between 2000-6000 x g for 1 minute. Place the Microspin column into a new, labeled Collection Tube. Repeat with second 500µl elution. \*Note: The eluates contain the purified antibodies. Do not discard!
- 8. Pool eluates from Step 7 and add 100µl of **LT Neutralization Buffer** 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.*
- \*IR-Tip: Each LigaTrap™ Ig Resin column can be used up to 10 times without significant loss of binding capacity
- 9. If the column will not be reused, it can be discarded. If column is to be reused, regenerate the column by adding 500μl of **LT Regeneration Buffer**. Vortex briefly for 15 seconds. Insert the Microspin column into a 2.0 mL Collection Tube and centrifuge between 2000-6000 x g for 1 minute.
- 10. To store resin add 500μl of **LT Storage Buffer.** Insert the Microspin column into a 2.0 mL Collection Tube. Centrifuge between 2000-6000 x g for 1 minute. Discard wash. Repeat with an additional two washes for a total of three washes. (3x500μl) Insert the bottom plug into the Microspin column, add 500μl of fresh **LT Storage Buffer** and store at 2-8<sup>2</sup> *C*.

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