

LigaTrap® Human IgM Purification Kit, 10 x 0.1 mL Spin Columns

Part # *LT-143KIT*

Kit Contents

Part #	Item	Quantity
LT-143-SC	Microspin Columns - centrifuge columns supplied with caps and plug. Each column contains 0.1 mL LigaTrap® Human IgM Purification Resin in PBS buffer with 0.05% sodium azide.	10
BU-131-FP	LigaTrap Sample Diluent 2.0	15 mL
BU-132-FP	LigaTrap Equilibration/Wash Buffer 2.0	250 mL
BU-123-FP	LigaTrap Elution Buffer	125 mL
BU-124-FP	LigaTrap Regeneration Buffer	50 mL
BU-125-FP	LigaTrap Neutralization Buffer	15 mL
BU-126-FP	LigaTrap Storage Buffer	50 mL
PL-057	2 mL Collection Tubes	80

Introduction

LigaTrap® Technologies has developed a novel, patented, series of affinity ligands specific for the purification of monoclonal and polyclonal immunoglobulins from various species. **LigaTrap® Human IgM Purification Resin** is engineered to purify high quality human IgM antibodies from recombinant and monoclonal sources. **Binding capacity for monoclonal human IgM is $\geq 15\text{mg/ml}$ resin.** The **LigaTrap® Human IgM Purification Kit** provides all the necessary reagents for fast, convenient micro-scale purification of human IgM in just 10 easy steps. Each kit contains 10 microspin columns prefilled with 0.1ml of **LigaTrap® Human IgM 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.

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 400µl of sample matrix (i.e. hybridoma supernatant or cell culture fluid) containing human IgM.
2. Add 100µl of **LigaTrap Sample Diluent 2.0** to the sample. Mix briefly by vortexing.

Purification

3. Equilibrate resin by adding 400µl of **LigaTrap 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 1000-3000 x g for 1 minute. Empty the buffer from Collection Tube. Repeat with two additional 400µl equilibration. Insert the bottom plug into the microspin column.
4. Transfer 400µl 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 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 400µl of the **LigaTrap Equilibration/Wash Buffer 2.0**. 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. (4x400µl)
7. Insert the washed microspin column into a **new, labeled** collection tube. Add 400µl of **LigaTrap Elution Buffer** 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 400µl elution. ****Note: The eluates contain the purified antibodies. Do not discard!***
8. Pool eluates from Step 7 and add 80µl (10-12% v/v of elution samples) of **LigaTrap 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.***
9. If the column will not be reused, it can be discarded. If column is to be reused, regenerate the column by adding 400µl of **LigaTrap Regeneration Buffer**. 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 400µl of **LigaTrap Storage Buffer**. 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. (3x400µl) Insert the bottom plug into the Microspin column, add 400µl of fresh **LigaTrap Storage Buffer** and store at 2-8° C.