Ordering Information

Description	Size (mL)	Part Number
Protein G Antibody Isolation Kit	20 samples	555010

Other related products:

Protein A Antibody Isolation Kit	20 samples	555000
Protein A Magnetic Particles	2, 5	544030/31
Protein G Magnetic Particles	2, 5	544040/41
Multi-6 Microcentrifuge Separator		472260
Solo Tube Separator		472270
15/50 mL Tube Separator		472250

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Protein G Magnetic Particles

Antibody Isolation Kit (20 Samples)

Product No. 555010

1. General Description

The Abraxis' superparamagnetic nanoparticles are coupled with a biomolecule, such as Protein G, and are utilized in the magnetic separation and isolation of antibodies from serum, cell culture supernatants, or ascites. The particles have a large surface area with high capture efficiencies.

2. Safety Instructions

Reagents contain 0.05-0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal.

3. Storage and Stability

The Protein G Magnetic Particle Kit should be stored in the refrigerator (4-8°C) prior to use. The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Do not freeze, dry, or centrifuge the particles as they may result in loss of binding activity and aggregation.

4. Test Principle

Protein G magnetic particles are incubated with the antibody solution and then separated by magnets. After the unbound particulates are washed from the particles, the bound antibodies are eluted from the particles using the elution buffer. The particles are then magnetically separated from the eluted solution, which is removed manually.

5. Warning and Precautions

-Do not freeze reagents.
-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
-Ensure that reagent bottle caps are tight after each use to prevent drying of reagents.
-Mistakes in handling the test can also cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit (or reagents), incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times, and/or short magnetic separation times.

6. Characteristics

Particle mean diameter: ~0.5 µm
Particle concentration: 5 mg/mL (10 mg in 2 mL)
Binding capacity: ≥ 60 µg rabbit IgG/mg of particles

7. Antibody Isolation

A. Materials Provided

1. Protein G magnetic particles, 5 mg/mL, 2 mL
2. Binding/Wash Buffer: Tris-Buffered-Saline with 0.05% Tween 20 detergent, 100 mL
3. Elution Buffer: 0.1 M Glycine pH 2.0, 5 mL
4. Neutralization Buffer: 1M Tris pH 8.0, 1 mL

B. Additional Materials (not provided with the kit)

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. 1.5 mL or 2.0 mL Eppendorf or microcentrifuge vials
3. Timer
4. Rotator
5. Distilled or deionized water
6. Vortex mixer
7. Solo or Multi-6 Microcentrifuge Separator (PN 472270; PN 472260)

C. Procedures

1. Add 100 µL (0.5 mg) of particles to 1 mL of binding buffer in each tube to wash particles.
2. Magnetically separate using a magnetic separator for 2 minutes or when the supernatant is clear.
3. Remove the supernatant and wash once more by adding 1 mL of binding buffer.
4. Repeat step 2 and remove the supernatant.
5. Resuspend particles by adding 450 uL of binding buffer.
6. Add 50 µL of serum or cell culture supernatant to the particles.

   Note: Sample volume can be modified according to user preference. If the sample volume is < 500µL, dilute it to a final volume of 500µL with Binding/Wash Buffer.
7. Gently mix using vortex or rotator for 30 minutes.
8. Magnetically separate using a magnetic separator for 2 minutes or when the supernatant is clear.
9. Remove supernatant and wash with 0.5 mL Binding/Wash buffer to remove unbound proteins.
10. Repeat steps 8 and 9 once more. Remove supernatant.
11. Add 100 µL of elution buffer to particles and mix well.
12. Incubate at room temperature for 10 minutes with occasional gentle mixing or vortex.
13. Separate for 2 minutes and remove the eluent to a new tube containing 15 µL of neutralization buffer.

9. Binding Capacities for IgG Proteins Table

Antibody binding affinity to Protein A and Protein G*

Species	IgG Class	Protein A	Protein G
Chicken egg	IgY	—	—
Cow	IgG	—	+
Dog	IgG	+	+
	IgM	+	—
Goat	IgG	+	+++
	IgM	—	—
Horse	IgG	+++	+++
Rabbit	IgG	+++	+++
	IgM	—	—
Rat	IgG	+	++
	IgM	—	—
Sheep	IgG	+++	+++
	IgM	—	—
Mouse	IgG1	+	++
	IgG2a	++	++
	IgG2b	++	++
	IgG3	+	++
	IgM	++	+
	IgA	++	++
Human	IgG1	+++	+++
	IgG2	+++	+++
	IgG3	—	+++
	IgG4	+++	+++
	IgA	+	—
	IgM	+	—
	IgE	+	—

— No binding; + weak binding; ++ moderate binding; +++ strong binding.
* Data obtained from Handbook of Affinity Chromatography by David S. Hage (ISBN 0824740572). Chapter 14 "Affinity Chromatography in Antibody and Antigen Purification" by Terry M. Phillips.