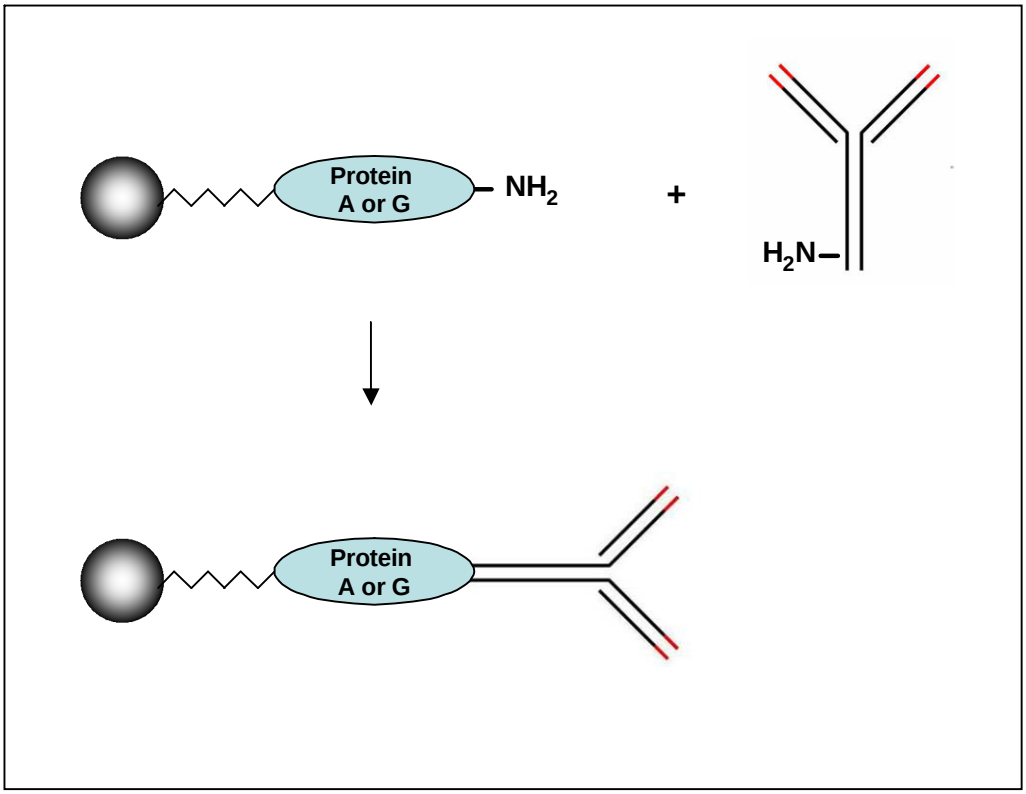
Protocol for purification of IgG molecules with SiMAG-Protein A or SiMAG-Protein G

Introduction:

This protocol describes a quick purification of IgG molecules from serum, ascites or cell culture supernatants with **SiMAG-Protein A** or **SiMAG-Protein G.**

Protein A/G are covalently coupled to a magnetic silica particles to provide an efficient method of purifying antibodies.

SiMAG-Protein A/G binds with high specifity to the Fc region of most IgG subclasses from different species (for details on affinity, see page 4).



Reagents:

- **Wash & Binding buffer (W&B buffer):**

  0.1 M sodium phosphate, 0.15 M NaCl, pH 7.5

- **Elution buffer:** 0.1 M glycine, 0.15 M NaCl, pH 2.8

Protocol:

1. Wash the SiMAG-Protein A / G beads (10 mg / ml) three times with 1 ml W & B buffer by magnetic separation and resuspended the beads in 0.5 ml W & B buffer by vortexing.

2. Mix the IgG containing sample 1:1 with the W & B buffer (final volume not greater as 1.5 ml and not more than 5 mg IgG).

3. Add the SiMAG-Protein A / G beads (10 mg / ml) to the IgG containing sample, incubated with mixing constantly for 15 minutes at room temperature (RT).

4. Collect the beads for 30 seconds with a magnet, remove and discard the supernatant.

5. Add 1.5 ml W & B buffer vortex for 5 seconds, collect the beads for 30 seconds with the magnet, remove and discard the supernatant and repeat the washing step two times.

6. Add to the beads 0.2 - 0.5 ml Elution buffer, vortex and incubate for 10 minutes at RT in a thermo-mixer or shake the tube intermediately.

7. Collect the beads with the magnet and transfer the solution with the eluted IgG in a new tube. If the solution not clear, repeat the step.

8. Immediately neutralize the eluting IgG by adding 0.1 ml 1M Tris-HCl, pH 8.0. Dialyze against 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2 and store at 4°C with a preservative (0.02 % sodium azide) or lyophilize.

Note:

For antibodies that are sensitive to a low pH conditions, an alternative Elution buffer can be used:

6.0 M Urea

5.0 M Potassium iodide

3.0 M Potassium chloride

1.0 M Ammonium thiocyanate

0.1 M Tris-acetate, 2.0 M NaCl, pH 7.7

2.0 M Trichloroacetic acid-NaOH, pH 7.0

Isotype Elution for Purification of mouse IgG subclasses:

Protein A / G interacts with all mouse IgG subclasses, the affinity of the interaction varies (depended on pH and salt concentration). Therefore it is possible to elute the bounded IgG subclasses sequentially with following isotype elution buffers (step 6):

Isotype Elution buffer 1:	0.1 M potassium phosphate, pH 6.0
Isotype Elution buffer 2:	0.1 M sodium citrate, pH 5.5
Isotype Elution buffer 3:	0.1 M sodium citrate, pH 5.0
Isotype Elution buffer 4:	0.1 M sodium citrate, pH 4.5
Isotype Elution buffer 5:	0.1 M sodium citrate, pH 3.5
Isotype Elution buffer 6:	0.1 M sodium citrate, pH 3.0

The affinity of SiMAG-Protein A and SiMAG-Protein G for immunoglobulins from different species

Species	Subclass	SiMAG-Protein A	SiMAG-Protein G
Bovine	IgG1 IgG2	weak strong	strong strong
Chicken	IgG	none	none
Dog	IgG	strong	weak
Goat	IgG1 IgG2 IgM	weak strong none	strong strong none
Guinea pig	IgG1 IgG2	strong strong	strong strong
Horse	IgG	weak	strong
Human	IgG1 IgG2 IgG3 IgG4 IgA2 IgM	strong strong weak strong weak weak	strong strong strong strong none none
Mouse	IgG1 IgG2a IgG2b IgG3 IgM	weak strong strong strong none	weak strong strong strong none
Pig	IgG	strong	weak
Rabbit	IgG IgM	strong none	strong none
Rat	IgG1 IgG2a IgG2b IgG2c IgM	weak none none strong none	weak strong weak strong none
Sheep	IgG1 IgG2 IgM	weak strong none	strong strong none