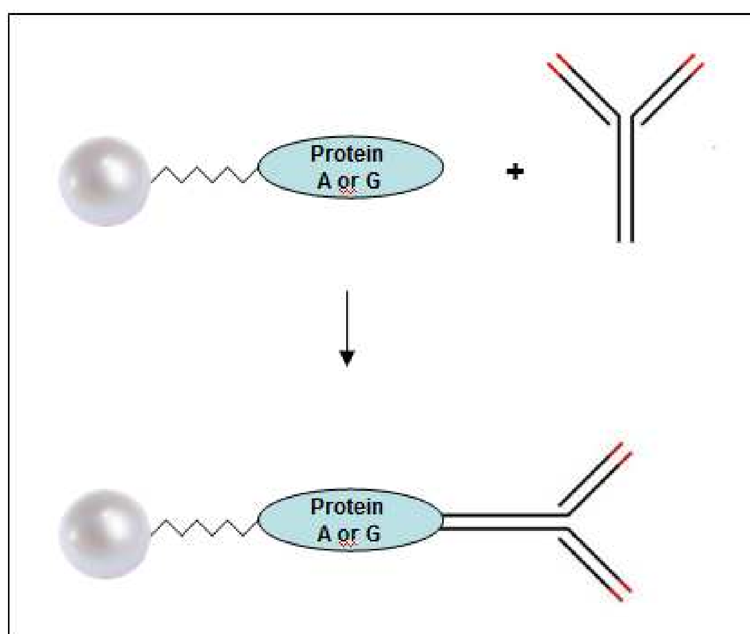
Protocol for purification of IgG molecules with beadBALL-Protein A or beadBALL-Protein G

Introduction:

This protocol describes a quick enrichment procedure of IgG molecules from serum, ascites or cell culture supernatants by means of **beadBALL-Protein A** or **beadBALL-Protein G.**

Protein A / G is covalently coupled to the microspheres to provide an efficient tool for purifying different IgG antibodies.

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



Equipment and reagents:

- **beadBALL-Protein A / G**
  (10 mg/ml in PBS, 0.05% sodium azide)

- **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

- **Microcentrifuge**

Protocol:

1. Transfer 1 ml **beadBALL-Protein A** or **beadBALL-Protein G** microspheres in a 2 ml microcentrifuge tube, add 1 ml **W & B buffer** and centrifuge for 1 minute at 500 x g. Remove the supernatant and repeat this step twice. Completely resuspend the microspheres in 0.5 ml **W & B buffer**.

2. Mix the IgG containing sample 1:1 with the **W & B buffer** (final volume < 1.5 ml, maximal IgG amount 5 mg).

3. Add the **beadBALL-Protein A / G** microspheres to the IgG containing sample, incubated by constantly mixing for 15 minutes at room temperature.

4. Spin down, remove and discard the supernatant.

5. Add 1.5 ml **W & B buffer** resuspend thoroughly, spin down, remove and discard the supernatant and repeat the washing step two times.

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

7. Spin down and transfer the solution with the eluted IgG in a new tube. If the solution is not clears, repeat this step.

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

Note:

An alternative **Elution buffer** can be used for antibodies which are sensitive to low pH conditions:

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 of mouse IgG subclasses:

Protein A / G interacts with all mouse IgG subclasses, the affinity of the interaction varies (depending 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

Affinity of beadBALL-Protein A and beadBALL-Protein G for immunoglobulins from different species

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