

# Immunoprecipitation Protocol Using Dynabeads® Protein A or Dynabeads® Protein G

- **Binding of Antibody (Ab)**

1. Completely resuspend Dynabeads by pipetting or rotating on a roller (5 min).
2. Transfer 50  $\mu$ l Dynabeads to a tube, place on magnet and remove supernatant.
3. Remove tube from magnet and resuspend the Dynabeads in 200  $\mu$ l Ab Binding & Washing Buffer containing your Ab of choice. (*Typically 1 - 10  $\mu$ g Ab, the optimal amount needed will depend on the individual Ab used*).
4. Incubate 10 minutes with rotation at room temperature.
5. Place tube on magnet and remove supernatant.
6. Remove tube from magnet and wash the Dynabeads-Ab complex by resuspending in 200  $\mu$ l Ab Binding & Washing Buffer.

- **Immunoprecipitation of Antigen (Ag)**

7. Place tube on magnet and remove supernatant
8. Add your Ag-containing sample (typically 100 - 1,000  $\mu$ l) to the Dynabeads-Ab complex and gently resuspend by pipetting.
9. Incubate 10 minutes at room temperature with rotation.
10. Place tube on magnet, transfer supernatant to a clean tube.
11. Wash the Dynabeads-Ab-Ag complex 3 times, using 200  $\mu$ l Washing Buffer for each wash. Mix gently by pipetting.
12. Resuspend the Dynabeads-Ab-Ag complex in 100  $\mu$ l Washing Buffer and transfer the suspension to a clean tube. Place tube on magnet and remove supernatant.

- **Elution of Ab/Ag complex** (*alternatives A: denaturing or B: non-denaturing*)

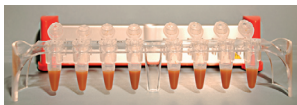
- A. Gently resuspend the Dynabeads-Ab-Ag complex in 20  $\mu$ l Elution Buffer. Add 10  $\mu$ l NuPAGE® LDS Sample Buffer / NuPAGE® Reducing Agent mix and incubate 10 minutes at 70°C. Place tube on magnet and load supernatant/sample onto a gel. (*Alternatively, the Dynabeads-Ab-Ag complex can be resuspended in the SDS sample buffer of your choice and heated as per your standard protocol prior to gel loading.*)
- B. Gently resuspend the Dynabeads-Ab-Ag complex in 20  $\mu$ l Elution Buffer. Incubate 2 minutes at room-temperature. Place tube on magnet and transfer supernatant/sample to a clean tube.

See product insert for detailed protocols.

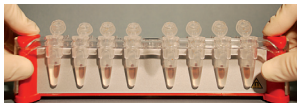
Species	Ig subclass	Dynabeads® Protein A	Dynabeads® Protein G
Goat	IgG1	+	+++
	IgG2	+++	+++
Mouse	IgG1	+	+++
	IgG2a	+++	+++
	IgG2b	+++	+++
	IgG3	+++	+++
	IgM	+	+
Rabbit	IgG	+++	+++
Rat	IgG1	+	+
	IgG2a	-	+++
	IgG2b	-	+
	IgG2c	+++	+
Sheep	IgG1	+	+++
	IgG2	+++	+++

*For a complete Ig-subclass matching guide, please see the product insert*

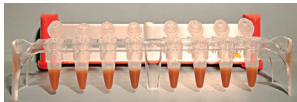
## Tips for using the magnet (DynaMag™-2, Cat. no. 123-21D)



The removable clear plastic rack can be used to hold your sample tubes both on and off the magnet. We recommend that you hold the rack/magnet with one hand to prevent it from accidentally tipping while pipetting.



Use both hands to remove the clear plastic rack from the magnet base. Grasp the rack from both ends and lift while pressing down with your fingers on the red tabs.



Mixing is done with the rack off the magnetic base. With the tubes still in the rack, simply pipette up-and-down to mix.