PURYMag Protein G

P9502

Storage

Store at 4-8 °C. Do not freeze

Stable for 1 year from purchase when stored properly.

Contents

- Product manual
- PURYMag Protein G, 30mg/ml

Introduction

PURYMag Protein G are Protein G conjugated magnetic beads. The Protein G is covalently coupled to their surface makes most of the binding sites sterically available for binding of IgG. Attribute to their uniform size, narrow size distribution, short magnetic separation time, and unique surface coating, PURYMag Protein G exhibit significantly low non-specific binding and fast magnetic separation.

Form

PURYMag Protein G contains 30 mg/mL of beads in phosphate buffered saline (PBS), pH 7.4, with 0.01% Tween™ 20 and 0.01% Proclin as a preservative.

Specifications

Binding capacity	8 - 10 ug Human IgG/mg of beads
Format	Beads in Suspension, 30mg/ml
Particle size	3 um
Concentration	30mg/ml

Required Materials & Buffers

- Magnetic Seperation Rack
- Sample Mixer allowing tilting and rotatin of tubes.
- Cell lysis buffer (e.g. Cell Extraction Buffer or NP-40 Cell Lysis Buffer)
- PBS pH 7.4 with and without 0.02% Tween™ 20
- 50 mM glycine pH 2.8 (elution buffer)
- LDS Sample Buffer and Sample Reducing Agent (elution buffer)

Note: For standard immunoprecipitation use PBS for antibody binding and washing steps. Other possible buffers include alternative phosphate buffers, HEPES, Tris, and lysis buffer (e.g. RIPA, NP40). Elution buffer may also be substituted by alternative low pH, high pH, or high salt buffers, depending on the application.

Procedure for Immunoprecipitation

This protocol provides a general procedure for immunoprecipitation. Optimization may be required for each antibody and target antigen. The protocol uses 50 µL of PURYMag Protein G, but may be scaled up or down as required.

Lyse cells

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of NP-40 Cell Lysis Buffer.

Note: For sensitive proteins and phosphorylation studies, perform the isolation protocol and elution at 4°C to avoid protein complex dissociation and minimize enzymatic activity.

Prepare PURYMag Protein G magnetic beads

1. Resuspend PURYMag Protein G beads in the vial (vortex >30 seconds or tilt and rotate 5 minutes).
2. Transfer 50 µL (1.5 mg) of PURYMag Protein G beads to a tube.
3. Place the tube on the magnetic rack to separate the beads from the solution, and remove the supernatant.
4. Remove the tube from the magnet.
5. Proceed directly to “Bind antibody”.

Bind antibody

1. Add your antibody (typically 1–10 µg) diluted in 200 µL PBS with Tween™ 20, to the PURYMag Protein G beads from step 4 in “Prepare PURYMag Protein G beads”. The optimal amount of Ab depends upon the individual Ab used.
2. Incubate with rotation for 10 minutes at room temperature.

Note: An incubation time of 10 minutes for immunoprecipitation is sufficient for most applications. Increasing the incubation time to 20–120 minutes can increase yield, particularly for low affinity antibodies, but may increase non-specific binding.

3. Place the tube on the magnetic rack and remove the supernatant.
4. Remove the tube from the magnetic rack and resuspend the magnetic bead-Ab complex in 200 µL PBS with Tween™ 20. Wash by gentle pipetting.

Note: Ab-conjugated PURYMag Protein G beads can be stored in PBS (pH 7.4) with 0.01–0.1% Tween™ 20 to prevent aggregation.

5. Proceed to “Immunoprecipitate target antigen”.

Note :To avoid co-elution of your antibody, crosslink your antibody to the PURYMag Protein G beads before immunoprecipitation. Use the crosslinking reagent BS3(Sulfo-DSS).

Note: For low-affinity antibodies, pre-incubate the sample and antibody prior to bead capture to improve binding kinetics for the antibody and minimize non-specific binding. This approach is also recommended when working with protein/nucleic acid complexes, e.g. ChIP.

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Immunoprecipitate target antigen

1. Place the tube (from step 4 of "Bind antibody") on the magnetic rack and remove the supernatant.
2. Add your sample containing the antigen (Ag) (typically 100–1000 µL) and gently pipette to resuspend the magnetic bead-Ab complex.
3. Incubate with rotation for 10 min at room temperature to allow Ag to bind to the magnetic bead-Ab complex.

Note: Depending on the affinity of the antibody, it may be necessary to increase incubation times for optimal binding.

4. Place the tube on the magnetic rack. Transfer the supernatant to a clean tube for further analysis, if desired.
5. Wash the magnetic bead-Ab-Ag complex 3 times by gentle pipetting using 200 µL of Washing Buffer for each wash. Place the tube on the magnetic rack and remove the supernatant between each wash.
6. Resuspend the magnetic bead-Ab-Ag complex in 100 µL of Washing Buffer and transfer the bead suspension to a clean tube to avoid co-elution of proteins bound to the tube wall.

Note: To store the immunoprecipitated protein, add elution buffer and sample buffer, then freeze the magnetic bead-Ab-Ag complex. For subsequent analysis of the sample, thaw and continue with the elution protocol.

7. Proceed to "Elute target antigen".

Elute target antigen: Denaturing elution

1. Place the tube (from step 6 of "Immunoprecipitate target antigen") on the magnetic rack and remove the supernatant.
2. Add 20 µL of Elution Buffer, and 10 µL of Laemmli Sample Buffer (4X),Reducing (Cat No, L1100).
3. Gently pipette to resuspend the magnetic bead-Ab-Ag complex.
4. Heat for 10 minutes at 70°C.
5. Place the tube on the magnetic rack and load the supernatant/sample onto a gel.

Note: As an alternative, the magnetic bead-Ab-Ag complex can be resuspended in a different sample buffer of your choice (e.g. SDS sample buffer). Follow the recommended temperatures and heating times for these buffers prior to gel loading.

Elute target antigen: Non-denaturing elution

1. Place the tube (from step 6 of "Immunoprecipitate target antigen") on the magnetic rack and remove the supernatant.
2. Add 20 µL Elution Buffer and gently pipette to resuspend the magnetic bead-Ab-Ag complex. Avoid foaming.
3. Incubate with rotation for 2 minutes at room temperature to dissociate the complex.
4. Place the tube on the magnetic rack and transfer the supernatant containing eluted Ab and Ag to a clean tube. If the eluted protein is to be used for functional assays or stored, the pH of the eluate can be adjusted by adding 1 M Tris, pH 7.5.

Binding Strength of Protein A and G by Species and Subclass

Ig origin	Affinity for Protein A	Affinity for Protein G
Human IgG1, 2, 4	+++	+++
Human IgD	-	-
Human IgA, E, M	+	-
Human IgG3	+	+++
Mouse IgG1	+	+++
Mouse IgG2, 2b, 3	+++	+++
Mouse IgM	+	+
Rat IgG1	+	+
Rat IgG2a	-	+++
Rat IgG2b	-	+
Rat IgG2c	+++	+
Bovine IgG1	+	+++
Bovine IgG2	+++	+++
Chicken IgY	-	-
Dog IgG	+++	+
Goat IgG1	+	+++
Goat IgG2	+++	+++
Guinea Pig IgG	+++	+
Hamster	+	NA
Horse IgG	+	+++
Monkey IgG	+++	+++
Porcine IgG	+++	+++
Rabbit IgG	+++	+++
Sheep IgG1	+	+++
Sheep IgG2	+++	+++

Related Products

Product Name	Cat No
NP-40 Lysis Buffer (2X)	N1200
West-Q Pico Dura ECL Solution	W3653
West-Q Femto ECL Solution	W3680

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