

★ Storage

Store at 4-8°C. Do not freeze

★ Contents

- Product Manual
- Puredown Protein A/G-Agarose

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

★ Shipping Condition

Ship with ice pack.

★ Introduction

Protein A/G contains four Fc binding domains from Protein A and two from Protein G. Protein A/G binds to all human IgG subclasses, making it the ideal choice for purification of polyclonal or monoclonal IgG antibodies whose subclasses have not been determined. Protein A/G also binds well to all mouse IgG subclasses but does not bind mouse IgA, IgM or serum albumin. This makes Protein A/G an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses, without interference from IgA, IgM and murine serum albumin. Individual subclasses of mouse monoclonals are likely to have a stronger affinity to the chimeric Protein A/G than to either Protein A or Protein G. Puredown Protein A/G is covalently coupled to highly cross-linked agarose bead. This product is provided preblocked with fatty acid free bovine serum albumin and have been extensively washed to reduce non-specific binding. Formulations are ready to use and 10ul of agarose per 1ug of primary antibody can be used in standard immunoprecipitation procedures.

★ Formulations

Puredown Protein A/G-Agarose is supplied in a volume of 1.5ml consisting of 0.5ml Protein A/G agarose suspended in 0.08% sodium azides/PBS.

★ Specificity

Puredown Protein A/G-Agarose is suitable for immunoprecipitation of human IgG₁, IgG₂, IgG₃, and IgG₄, mouse IgG₁, IgG_{2a}, IgG_{2b} and IgG₃, rabbit, rat, cow, horse, sheep, goat, and guinea pig polyclonal antibodies.

★ Specifications

Ligand density: ~6 mg Protein A and ~2mg Protein G/ml gel
 Binding capacity: > 18-35mg/ml Human IgG
 Bead structure: 4% cross-linked agarose
 Bead size range: 45-185nm

★ Procedure

This procedure is for immunoprecipitation assay using Puredown products. These are basic guidelines and may need to be modified for your particular application.

1. Preclear lysate or media by adding ~1.0ug of control IgG (normal mouse, rat, rabbit or goat IgG, corresponding to the host species of the primary antibody), together with 10ul of resuspended volume of Puredown Protein A/G-Agarose.

Note: This step is optional.

2. Incubate at 4°C for 1 hour.
3. Centrifuge at 2,500 rpm for 15 minutes at 4°C.
4. Transfer supernatant to a fresh 15ml conical centrifuge tube on ice.
5. Determine total protein of the supernatant.
6. Transfer 1mg of total protein and 1ug of purified primary antibody (optimal antibody concentration should be determined by titration) to a 1.5ml micro-centrifuge tube.
7. Incubate for 1 hour at 4°C.
8. Add 10ul of resuspended volume of Puredown protein A/G-Agarose.
9. Cap tubes and incubate at 4°C on a rocker platform or rotating device for 1 hour to overnight.
10. Centrifuge at 2,500 rpm for 15 minutes at 4°C.
11. Carefully aspirate and discard supernatant.
12. Wash pellet 4 times with either 1.0ml of ice-cold cell lysis buffer or PBS, each time repeating centrifugation step above.
13. After final wash, resuspend pellet in 40ul of 2X Laemmli sample buffer.
14. Boil samples for 5minutes. Unused samples may be frozen.
15. Centrifuge at 2,500 rpm for 15 minutes at 4°C.
16. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

★ Related Product

Product Name	Cat No
Laemmli Sample Buffer (4X), Reducing	L1001
NP-40 Cell Lysis Buffer (2X)	N1200
10X PBS Buffer	P2100
Xpert Protease Inhibitor Cocktail (100X)	P3100
Xpert Phosphatase Inhibitor Cocktail (100X)	P3200
Puredown Protein G-Agarose	P9202
Puredown Protein A/G-Agarose	P9203
PureSelect Protein A-Agarose	P9301
PureSelect Protein G-Agarose	P9302
PureSelect Protein A/G-Agarose	P9303
RIPA Cell Lysis Buffer (1X), with EDTA	R4100
RIPA Cell Lysis Buffer with Triton (1X), without EDTA	R4200