Puredown Protein A-Agarose

P9201

Storage

Store at 4-8°C. Do not freeze

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Shipping Condition

Ship with ice pack.

Introduction

Protein A is a cell wall component and four high affinity binding sites capable of binding specificaly to the Fc region of immunoglobin molecules from several species. The Protein A molecule is heat-stable and retains its native conformation when exposed to denaturing reagents such as 4M urea, 4M thiocynate and 6M guanidine hydrochloride.

Puredown Protein A 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 primary antibody can be used in standard immuno-precipitaion procedures.

Formulations

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

Specificity

Puredown Protein A-Agarose is suitable for immunoprecipitation of human IgG_1, IgG_2, IgG_4, mouse IgG_{2a}, IgG_{2b}, and IgG_3, rabbit, rat, sheep, goat, and guinea pig polyclonal antibodies

Specifications

Ligand density: ~6 mg Protein A/ml drained gel
Binding capacity: > 35 mg/ml Human IgG
Bead structure: 4% cross-linked agarose
Bead size range: 45-165nm

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-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-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	L1100
NP-40 Cell Lysis Buffer (2X)	N1200
RIPA Lysis Buffer (1X) with EDTA	R4100
10X PBS Buffer	P2100
Xpert Protease Inhibitor Cocktail (100X)	P3100
Xpert Phosphotase 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 (1X) without EDTA	R4200

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com