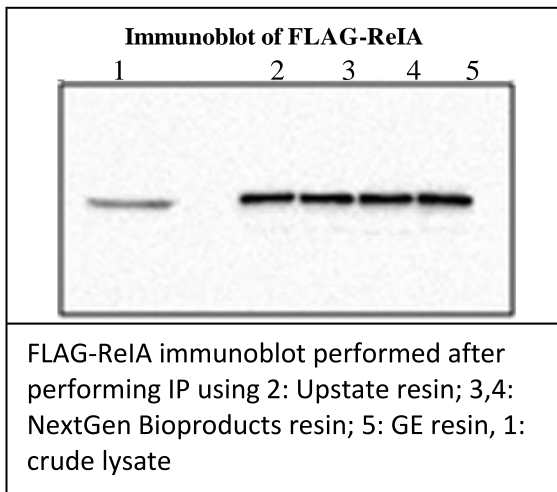
Product Name	Cat. No.	Pack Size
Protein AG^{PLUS} Agarose	**# BB-PAG001PA**	**0.5 ml Packed Bead Volume**
	# BB-PAG001PB	**1 ml Packed Bead Volume**
	# BB-PAG001PC	**2 ml Packed Bead Volume**
	# BB-PAG001PD	**5 ml Packed Bead Volume**
	# BB-PAG001PE	**10 ml Packed Bead Volume**

Protein AG has high affinity regions that are specifically bonded to the Fc region of the immunoglobulins. Protein AG is not as pH-dependent as Protein A alone, but otherwise has the additive properties of Protein A and G. Protein AG binds to all human IgG subclasses, making it the ideal choice for purification of polyclonal or monoclonal IgG antibodies whose subclass identities have not been determined.



FLAG-ReIA immunoblot performed after performing IP using 2: Upstate resin; 3,4: NextGen Bioproducts resin; 5: GE resin, 1: crude lysate

Protein AG^{PLUS} Agarose resin consists of Recombinant Protein AG (≥ 5 mg Protein AG/ ml resin) covalently bound to crosslinked activated agarose beads. It provides a very stable bond that can greatly minimize leakage of the Protein AG allowing for reuse of the affinity resin in several purification steps. The resin works well in batch or column purifications.

- Antibody binding efficiency was tested, as high as almost **17 mg per ml** of Protein AG^{PLUS} Agarose Resin.

This product was supplied as 50% suspension of Protein AG Agarose Resin in aqueous ethanol when packed, and the settled bead volume is X ml.

TECHNICAL SPECIFICATIONS

BEAD GEOMETRY & SIZE	: Spherical, ~ 50 - 150 µm diameter
CROSSLINKED	: Yes
BEAD CROSSLINKING %	: 6%
ACTIVATING GROUP	: Carbonyl
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% aqueous ethanol
STORAGE TEMPERATURE	: 4°C to 8°C. **DO NOT FREEZE.**

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A. Antibody Purification using Protein AG^{PLUS} Agarose Beads:

1. Take 1 ml bed volume of protein AG^{PLUS} agarose beads stored in 20% Ethanol
2. Wash the beads thrice with 1X PBS (pH-7.2-7.4)
3. Incubate protein AG beads with 5 ml antisera and 5 ml 1X PBS (pH-7.2-7.4) for 16h at 4°C in a rotating shaker
4. After 16hr load the beads in column followed by washing with 1X PBS (pH-7.2-7.4) for at least 3 times. Each time 10ml of 1X PBS to be used.
5. Elute the bound antibodies by 100 mM Glycine (pH-2.8) with 0.1% Tween 20
6. Collect 1.76 ml of aliquots into a 2 ml microcentrifuge tubes containing 40µl 3M Tris-HCl (pH-8.8) and 200 µl of 3M KCl or NaCl
7. Immediately mix up each aliquot thoroughly just after collection (2 ml total volume)
8. Measure the protein content of fraction by measuring OD at 280 nm or by Bradford method at 595 nm.
9. Analyze the fractions in SDS PAGE
10. If necessary, fractions can be pulled and dialyze against appropriate buffer and preserved accordingly in presence of 50% glycerol at -20°C.

B. Immunoprecipitation using Protein AG^{PLUS} Agarose beads

Make the Extract from RAW cells

1. Remove media from the plate. Add 1 ml PBS + 1 mM EDTA
2. Scrape cells and transfer to 1.5 µl centrifuge tube; spin down in microfuge for 30 sec. Remove supernatant.
3. Make cytoplasmic extract by adding 400 µl cytoplasmic extraction (CE) buffer and resuspend. (CE=10mM HEPES-KOH pH7.9, 250mM NaCl, 0.5% NP-40, 0.2% Tween 20, 1mM EDTA with freshly added Protease Inhibitor cocktail, 2 mM DTT).
   [If working with phospho protein addition of 1 mM PMSF, 10 mM NaF, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4 is needed].
4. Let sit in ice for 2 min. and vortex 1 min at setting 7-8.
5. Spin down lysate for 30 sec and remove cytoplasm supernatant and transfer to a fresh tube (can either discard or save the nuclear extract for other work).
6. Normalize extracts using Bradford assay.

Immunoprecipitation steps

1. Pre-clear lysate for 30 min with 5 µl (per 100-500 µl extract, depending on protein content) protein AG beads, at 4°C on rotor (beads should be 50% slurry in CE buffer).
2. Spin down beads for 2 min at 5000 rpm at RT (but keep on ice otherwise)
3. Remove pre-cleared 100 µl lysate and transfer to a fresh tube. [Step-1, 2, 3 is optional]

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4. Add 1 µg of antibody to lysate and let rotate for 1 hour at 4°C.

5. Add 7 µl protein AG*PLUS* beads/100-1000 µl (depending on protein content) extract and let rotate at 4°C for 1 hour. *[Binding in diluted condition is preferred]*

6. Spin for 30s to collect the beads. Can save the supernatant (to check the IP efficiency)

7. Wash beads twice with CE buffer (500 µl each).

8. Boil the beads with SDS-PAGE loading buffer, spin down and process for gel running and Western Blot.

[For any more technical assistance please communicate to **contact@nextgenbioproducts.com**]