Product Name	Cat. No.	Pack Size
Protein G^{PLUS} Agarose	**# BB-PG001PA**	**0.5 ml Packed Bead Volume**
	# BB-PG001PB	**1 ml Packed Bead Volume**
	# BB-PG001PC	**2 ml Packed Bead Volume**
	# BB-PG001PD	**5 ml Packed Bead Volume**
	# BB-PG001PE	**10 ml Packed Bead Volume**

Protein G has high affinity regions that are specially bonded to the Fc region of the Immunoglobulins (especially IgGs of different species). Protein G agarose beads is ideal for polyclonal IgG purification from mouse, human, goat, sheep serum, including human IgG3 and mouse IgG1 isotypes.

Protein G^{PLUS} Agarose resin consists of Recombinant Protein G **(≥ 5 mg Protein G /ml resin)** covalently coupled to cross-linked activated agarose beads. It provides a very stable bond that can greatly minimize leakage of the Protein G allowing for reuse of the affinity resin in several purification steps. The resin works well in batch or column purifications. The product is also suitable for Immunoprecipitation (IP) procedures.

This product is supplied as a suspension as 50% suspension of Protein G^{PLUS} Agarose Resin in 20% ethanol. The settled bead volume is as per product pack volume.

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.**

Shelf life18 months under proper storage & handling conditions.

For Research Use Only. Not For Use in Diagnostic Procedures

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

1. Take 1 ml bed volume of protein G^{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 16h 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 40 µl 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 G^{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 = 10 mM 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.

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Protocol: Antibody Purification using Protein G^{PLUS} Agarose Beads

Immunoprecipitation steps

1. Pre-clear lysate for 30 min with 5 µl (per 100-500 µl extract, depending on protein content) Protein G^{PLUS} 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]
4. Add 1 µg of antibody to lysate and let rotate for 1 hour at 4°C.
5. Add 7 µl protein G^{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 at **contact@nextgenbioproducts.com**]