TrueBlot® Protein G Magnetic Beads – PG00-18

Rockland's TrueBlot® Protein G Magnetic Beads are uniform, non-aggregating, super-paramagnetic beads coupled with a biomolecule, such as Protein G. These beads are specifically designed, tested and quality controlled for isolation and purification of antibodies, and immunoprecipitation methods using manual or automatic platforms. Protein G, attached to magnetic bead surface, can bind to antibodies from many different species, enabling purification of antibodies from crude extracts. Immunoprecipitation assays with Protein G magnetic beads result in high capture efficiencies and high yield of target antigen. Protein G magnetic beads are stable, pre-blocked beads that provide highly purified product.

I. PRODUCT CONTENTS

Contents	Catalog Number	Size
TrueBlot® Protein G Magnetic Beads	PG00-18-2	2 mL
TrueBlot® Protein G Magnetic Beads	PG00-18-5	5 mL

II. PRODUCT CHARACTERISTICS

Bead Mean Diameter	0.5 µm
Bead Concentration	5 mg/mL
Binding Capacity	≥ 80 µg rabbit IgG/mg of beads

III. STORAGE CONDITIONS

The Protein G Magnetic Beads should be stored in the refrigerator (2-8°C). The reagent must be allowed to reach room temperature (20-25°C) before use and may be used until the expiration date (one year from date of opening). Do not freeze, dry, or centrifuge the beads as they may result in loss of binding activity and aggregation.

IV. INTRODUCTION

TrueBlot® Protein G magnetic beads can be used for separation and purification of antibodies from serum or antibody-labeled components, as well as for immunoassays, immunoprecipitation, co-immunoprecipitation and immunoprecipitation Western blots. For antibody purification, Protein G magnetic beads are incubated with the antibody solution and then separated by magnets. After the unbound particulates are washed from the beads, the bound antibodies are eluted from the beads using the elution buffer. The beads are then magnetically separated from the eluted solution, which is removed manually. For immunoprecipitation, target specific antibody is incubated with cell lysate. Protein G magnetic beads are then incubated with antigen-antibody complex. The unbound material is washed from the beads and the purified protein is eluted using the elution buffer. The samples are then resolved by SDS-PAGE and analyzed by Western blotting.

V. REQUIRED EQUIPMENT AND REAGENTS

- Magnetic Separators (Cat # TMS-15-50; TMS-06; TMS-32)
- Mixer for tilting and rotation of tubes
- Binding/wash Buffer: TBS-0.05% Tween 20 or PBS-0.05% Tween

- IP Lysis Buffer
- Elution Buffer: 0.1 M Glycine pH 2.0
- Neutralization Buffer: 1M Tris-HCl pH 8.0
- Primary antibody
- Antigen sample

VI. GENERAL CONSIDERATIONS

- Do not centrifuge, dry or freeze the magnetic beads, as this can cause the beads to aggregate and lose the binding capability.
- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- Ensure that reagent bottle caps are tight after each use to prevent drying of reagents.
- Use a mixer that provides tilting and rotation of the tubes to ensure that the beads do not settle in the tube.
- Avoid air bubbles (foaming) during pipetting.
- Use recommended volume of beads.
- Carefully follow the recommended volumes and incubation times.
- Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test reagents, incorrect pipetting sequence or inaccurate volumes of the reagents, too short incubation times, and/or short magnetic separation times.

VII. ANTIBODY ISOLATION PROTOCOL

- Add 100 µL (0.5 mg) of beads to 1 mL of Binding Buffer in each tube to wash beads.
- Magnetically separate the beads using a magnetic separator for 2 minutes or when the supernatant is clear.
- Remove the supernatant and wash once more by adding 1 mL of Binding Buffer.
- Magnetically separate the beads using a magnetic separator for 2 minutes or when the supernatant is clear and remove the supernatant.
- Resuspend beads by adding 450 µL of Binding Buffer.
- Add 50 µL of serum or cell culture supernatant to the beads.
  Note: Sample volume can be modified according to user preference. If the sample volume is < 500 µL, dilute it to a final volume of 500 µL with Binding/Wash Buffer.
- Gently mix using vortex or rotator for 30 minutes.
- Magnetically separate using a magnetic separator for 2 minutes or when the supernatant is clear.
- Remove supernatant and wash with 0.5 mL Binding/Wash Buffer to remove unbound proteins.
- Magnetically separate using a magnetic separator for 2 minutes or when the supernatant is clear.
- Remove supernatant and wash with 0.5 mL Binding/Wash Buffer.

- Add 100 µL of Elution Buffer to beads and mix well.
- Incubate at room temperature for 10 minutes with occasional gentle mixing or vortex.
- Separate for 2 minutes and remove the eluent to a new tube containing 15 µL of Neutralization Buffer.

VIII. IMMUNOPRECIPITATION PROTOCOL

Immunoprecipitation (IP) is performed by either adding the selected primary antibody to Protein G magnetic beads followed by adding the sample antigen, or by first forming an antigen-antibody complex and then incubating the complex with Protein G magnetic beads. Bound antigen-antibody complex is then eluted using different elution methods.

A. General guidelines

- The amount of antibody captured depends on the concentration of antibody and Protein G magnetic beads in the starting sample.
- For standard immunoprecipitation use PBS or TBS for antibody binding and washing steps. However, these may be substituted by other buffers of choice, such as phosphate buffers, lysis buffer (e.g. RIPA, NP40), or HEPES. The recommended elution buffer may also be substituted by alternative low pH-, high pH- or high salt buffers, depending on the target protein and downstream application.

B. Preparation of Immune Complex

Note: The amount of sample needed and the incubation time are dependent upon each specific antibody-antigen system and may require optimization by the user for maximum yield. The following protocol is for 2-10 µg of affinity-purified antibody and can be scaled up as needed.

- Combine cell lysate containing the antigen (typically 100- 1000 µL) with 2-10 µg of IP antibody per sample in a microcentrifuge tube. The suggested amount of total protein per IP reaction is 500-1000 µg, as determined by the BCA Protein Assay.
- Dilute the antibody/lysate solution to 500 µL with IP Lysis/Wash Buffer.
- Incubate for 1 hour at RT or overnight at 4ºC to form the immune complex (the optimal incubation time can be determined in a preliminary experiment).

C. Immunoprecipitation with Antigen-Antibody Complex

Note: To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or using a rotating platform.

- Place 100 µL (0.5mg) of Protein G Magnetic Beads into a 1.5 mL microcentrifuge tube.
- Add 1 mL of IP Lysis/Wash Buffer to the beads and gently vortex to mix.
- Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
- Add 1 mL of IP Lysis/Wash Buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic separator. Remove and discard the supernatant.
- Add the antigen-antibody (Ag-Ab) complex (from Section B) to the tube containing pre-washed magnetic beads and incubate at room temperature for 1 hour with mixing.

- Collect the beads with a magnetic separator, remove the unbound sample and save for further analysis if desired.
- Add 500 µL of IP Lysis/Wash Buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.
- Low-pH Elution: Add 100 µL of Elution Buffer to the tube. Incubate the tube at RT with mixing for 10 minutes. Magnetically separate the beads and save the supernatant containing the target antigen. To neutralize the low pH, add 15 µL of Neutralization Buffer for each 100 µL of eluate.
- Alternative Elution: Add 100 µL of Sample Buffer to the tube and heat the samples at 96-100ºC in a heating block for 10 minutes. Magnetically separate the beads and save the supernatant-containing target antigen.

Note: If elution under reducing conditions is desired, add DTT (to a final concentration of 50 mM) to the 1X Sample Buffer.

D. Immunoprecipitation with Antibody-Magnetic Bead Conjugate

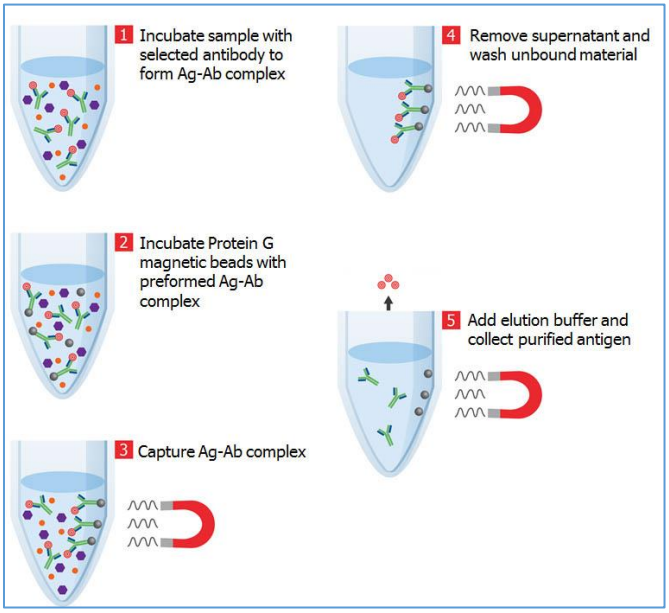
- Wash 100 µL (0.5mg) of Protein G Magnetic Beads as described above. Resuspend beads by adding 450 µL of IP Lysis/Wash Buffer.
- Add 2-10 µg (50 µL) of IP antibody to the bead slurry.
- Incubate the antibody-bead mixture for 1 hour at room temperature or 2–4 h at 4°C by gently mixing the mixture on a rotator.
- Place the tube into a magnetic stand to collect the antibody-bead mixture against the side of the tube. Remove and discard the supernatant.
- Add 1 mL of IP Lysis/Wash Buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic separator. Remove and discard the supernatant. Repeat this wash step one more time.
- After washing the beads and antibody mixture, add cell lysate containing the antigen (100–500 µL) and gently resuspend the lysate-bead/antibody conjugate mixture.
- Incubate the lysate-bead/antibody conjugate complex for 1 hour at room temperature or overnight at 4ºC under rotary agitation (the optimal incubation time can be determined in a preliminary experiment).
- Collect the beads with a magnetic separator, remove the unbound sample and save for further analysis if desired.
- Add 500 µL of IP Lysis/Wash Buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.
- Low-pH Elution: Add 100 µL of Elution Buffer to the tube. Incubate the tube at RT with mixing for 10 minutes. Magnetically separate the beads and save the supernatant containing the target antigen. To neutralize the low pH, add 15 µL of Neutralization Buffer for each 100 µL of eluate.
- Alternative Elution: Add 100 µL of Sample Buffer to the tube and heat the samples at 96-100ºC in a heating block for 10 minutes. Magnetically separate the beads and save the supernatant-containing target antigen.

  Note: If elution under reducing conditions is desired, add DTT (to a final concentration of 50 mM) to the 1X Sample Buffer.

IX. ASSAY SUMMARY

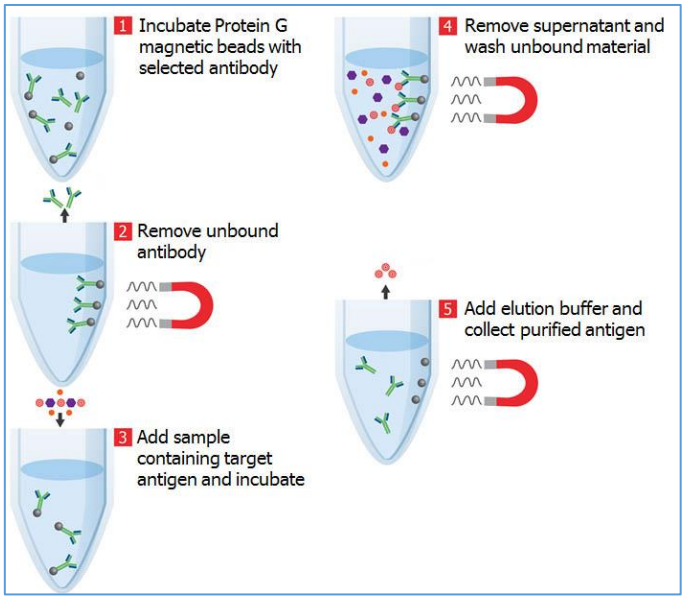
Immunoprecipitation with Antigen-Antibody Complex

- Incubate cell lysate with selected antibody for IP for 1-2 hours at RT or overnight at 4ºC to form Ag-Ab complex.
- Incubate Protein G magnetic beads with pre-formed Ag-Ab complex.
- Capture Ag-Ab complex using magnetic separator.
- Remove supernatant and wash beads twice with IP Lysis/Wash Buffer.
- Add Elution Buffer and elute purified antigen.



Immunoprecipitation with Antibody-Magnetic Bead Conjugate

- Incubate Protein G magnetic beads with the selected antibody for IP.
- Capture antibody-bead mixture using magnetic separator and remove unbound antibody.
- Add cell lysate containing target antigen and incubate for 30 minutes to 1 hour at RT.
- Remove supernatant and wash beads twice with IP Lysis/Wash Buffer.
- Add Elution Buffer and elute purified antigen.



X. RELATED PRODUCTS

General Use Magnetic Beads	Catalog #	Size (ml)
TrueBlot® Anti-Mouse IgG Magnetic beads (5mg/ml)	00-1811-20 00-1811-50	2 5
TrueBlot® Anti-Rabbit IgG Magnetic beads (5mg/ml)	00-1800-20 00-1800-50	2 5
TrueBlot® Anti-Goat IgG Magnetic Beads (5mg/ml)	00-1844-20 00-1844-50	2 5
TrueBlot® Protein A Magnetic Beads (5mg/ml)	PA00-18-2 PA00-18-5	2 5
TrueBlot® Streptavidin Magnetic Beads (5mg/ml)	S000-18-2 S000-18-5	2 5
TrueBlot® Biotin Magnetic Beads (5mg/ml)	B000-18-2 B000-18-5	2 5
TrueBlot® Nickel Magnetic beads (12.5mg/ml)	N000-18-2 N000-18-5	2 5

Magnetic Bead IP/Purification Kits	Catalog #	Size (tests)
TrueBlot® Protein A Magnetic Beads IP/Co-IP Kit	KBA-PA00-18	20
TrueBlot® Protein G Magnetic Beads IP/Co-IP Kit	KBA-PG00-18	20
Genomic DNA Magnetic Beads Purification Kit	KOA0912	100

Magnetic Separators	Catalog #	Size (tests)
15/50 ml Tube Magnetic Separator	TMS-15-50	6/3
Multi-6 Microcentrifuge Tube Magnetic Separator	TMS-06	6
Multi-32 Microcentrifuge Tube Magnetic Separator	TMS-32	16