

Product Information

PROTEIN G COATED HS 96-well Plates (clear)

Product Number **S 2063**

Storage Temperature 2 °C to 8 °C

TECHNICAL BULLETIN

Product Description

Protein G binds securely and specifically with the IgG's of many mammalian species (See Table 1). Binding of the antibody occurs only at the Fc region, leaving the Fab region free.

Protein G Coated HS 96-well Plates are coated with a genetically-engineered, truncated form of Protein G. It retains affinity for IgG, but lacks albumin and Fab binding sites, and membrane binding regions, thus reducing non-specific binding.¹⁻⁴

The Protein G Coated HS 96-well Plate is a versatile platform specifically designed for the capture of mammalian IgG. The IgG may be applied directly to the surface or captured as an antibody/antigen complex. Once captured, the antibody's antigen binding site is oriented away from the surface. This orientation allows for better performance in downstream assays.

Capture and detection of proteins with affinity ligands immobilized on solid supports has become common practice. Supports being used today are agarose, microparticles, membranes, and multiwell plates. ELISA, SDS-PAGE, Western Blot, MALDI mass spectroscopy, or affinity binding analysis may be used to analyze the antigen of interest. In the event that many samples containing antigen are to be analyzed, the Protein G Coated HS 96-well Plate offers greater speed and convenience over other methods. Protein G Coated HS 96-well Plates are also powerful tools in High Throughput Screening (HTS) applications. The plates may be used for general screening of antigen interaction, protein/protein interaction studies, protein/organic molecule interaction studies, signal transduction studies, and/or estimation of antigen content.

Component

The plate is supplied as a 96 well plate with clear sides and bottom.

Coating

Recombinant Protein G', from *Streptococcus sp.*, expressed in *E. coli*, is coated using 200 µl/well. The wells are pre-blocked for convenience at 275-300 µl/well.

Specificity

The plates are specific for mammalian IgG. See Table 1.

Sensitivity

Detection of 1 ng/well of a monoclonal antibody (isotype IgG1) alkaline phosphatase conjugate was observed in an ELISA format with p-Nitrophenyl Phosphate (pNPP) as a substrate.

Precautions and Disclaimer

- Regeneration and reuse of the plate is not recommended.
- This product is for laboratory use only, not for drug, household, or other use.

Storage/Stability

Store at 2 °C to 8 °C. If unopened, Protein G Coated HS 96-well Plates are stable for at least two years. If opened, the plate should be stored with desiccant at 2 °C to 8 °C and used within two weeks.

Procedures:

Binding and Elution of a mammalian IgG/antigen complex for SDS-PAGE Analysis:

Materials Required

- Protein G Coated HS 96-well Plates, Product No. S 2063
- Antibody to specific antigen of interest

- Cell lysate containing specific antigen of interest
1. Add antibody to cell lysate containing specific antigen.
 2. Allow cell lysate to incubate for 1-2 hours.
 3. Load up to 200 μ l of cell lysate containing the antibody/antigen complex per well.
 4. Cover with a 96-well plate cover or sealing tape.
 5. Allow the cell lysate to incubate in the plate for a minimum of one hour at 37 °C or 2 hours at room temperature. If incubating overnight, place at 4 °C.
 6. Remove the plate cover and wash the plate three times (300 μ l per well) with an appropriate wash buffer (e.g., 100 mM Tris buffered saline, pH 8.2, containing 2 mM MgCl₂, 0.5 % Tween, for Alkaline Phosphatase conjugates).

Note: Plate washing may be done with an automated plate washer or by hand with a multi-channel pipet. If washing by hand, remove any residual liquid by inverting the plate and gently patting it on lint-free paper towels.
 7. Elute the antigen by loading 30 μ l of a 2X sample buffer containing 4 % SDS, 20 % glycerol, 10 % 2-mercaptoethanol, 0.004 % bromophenol blue and 125 mM Tris HCl, pH 6.8 (Product No. S 3401).

Note: Any one of several common elution buffers may be used to elute antigen from the surface of the plate. We use the buffer described above for preparation of proteins for SDS-PAGE analysis, as described below.
 8. Seal the plate with sealing tape.
 9. Vortex for a minimum of 10 minutes, making certain that the buffer completely washes the sides of the wells. Caution: ensure that the buffer does not splash from the plate.
 10. Transfer the samples from the wells to clean microcentrifuge tubes using a micropipettor
 11. Load samples directly onto a SDS-polyacrylamide gel and run as per the manufacturer's recommendations.

12. Gels may be stained or Western Blot analysis may be performed using standard protocols.

ELISA Screening of FLAG Fusion Protein

Materials Required

- Protein G Coated HS 96-well Plate, Product No. S 2063
- Antibody for fusion protein or alternate tag
- ANTI-FLAG M2 Alkaline Phosphatase, Product No. A 9469
- Substrate for the detection of enzyme

Equipment Required

- Multi-channel (8- or 12-channel) pipet
 - Spectrophotometer capable of reading 96-well microtiterplates at 405 nm
1. Load up to 200 μ l of a 1 ng/ μ l to 10 ng/ μ l solution of antibody to fusion protein or alternate tag in an appropriate loading buffer (e.g., Tris buffered saline containing 1 % BSA, 0.5 % Tween 20).

Note: If working with a dual tagged fusion protein, for example FLAG[®] and c-Myc, capture the c-Myc antibody on the surface of the plate.
 2. Cover with a 96-well plate cover or sealing tape.
 3. Allow the antibody to incubate in the plate for a minimum of one hour at room temperature. If incubating overnight, place at 4 °C.
 4. Remove the plate cover and wash the plate three times (300 μ l per well) with an appropriate wash solution (e.g., Tris buffered saline containing 0.5 % Tween 20).

Note: Plate washing may be done with an automated plate washer or by hand with a multi-channel pipet. If washing by hand, remove any residual liquid by inverting the plate and gently patting it on lint-free paper towels.
 5. Apply up to 200 μ l of cell lysate or fusion protein solution to each well. Allow the lysate to incubate in the plate for a minimum of one hour at room temperature. If incubating overnight, place at 4 °C.
 6. Remove the plate cover and wash the plate three times (300 μ l per well) with an appropriate wash solution (e.g., Tris buffered saline containing 0.5 % Tween 20)

7. Apply 250 µl of the diluted ANTI-FLAG M2 alkaline phosphatase conjugate. Cover the plate with a 96-well plate cover or sealing tape. Allow the plate to incubate for a minimum of one hour at room temperature (18 °C to 26 °C).
8. Remove the plate cover and wash the plate three times (300 µl per well) with an appropriate wash solution.

1. Prepare any necessary substrate and/or stop solutions per product instructions (e.g., Product No. N 7653).
2. Apply up to 200 µl of the appropriate substrate per well.
3. Using a 96-well plate reader, record signal per substrate manufacturer's recommendations (e.g., 405 nm for pNPP).

Detection of Bound Fusion Proteins

Table 1.
Protein A, G, and L Capacities for Various Species

Species	Immunoglobulin	Binding – Protein A ⁵⁻⁸	Binding – Protein G ^{2-4,9}	Binding – Protein L ¹⁰
Human	IgG (normal)	++++	++++	++++
	IgG1	++++	++++	++++
	IgG2	++++	++++	++++
	IgG3	—	++++	++++
	IgG4	++++	++++	++++
	IgM	—	—	++++
	IgA	—	—	++++
	IgE	—	—	++++
	IgD	—	—	++++
	Fab	++	++	++++
	K light chains	—	—	++++
	L light chains	—	—	—
	ScFv	++	—	++++
Mouse	IgG1	+	++++	++++
	IgG2a	++++	++++	++++
	IgG2b	+++	+++	++++
	IgG3	++	+++	++++
Rat	IgG1	—	+	++++
	IgG2a	—	++++	++++
	IgG2b	—	++	++++
	IgG2c	+	++	++++
Bovine	IgG	++	++++	—
Cat	IgG	++++	—	N/A
Chicken	IgG	—	+	++
Dog	IgG	++++	++++	+
Goat	IgG	+/-	++	—
Guinea Pig	IgG	++++	++	++
Hamster	IgG	+	++	++++
Horse	IgG	++	++++	+/-
Pig	IgG	+++	+++	++++
Rabbit	IgG	++++	+++	+
Sheep	IgG	+/-	++	—

Table 2.
Troubleshooting Guide

Problem	Possible Cause	Solution
High Background	Crude cell lysate may contain enzymes capable of turning over substrate	Check a negative control, non-expressing cell lysate
	Antibody titer too high	Increase the dilution of the secondary antibodies
	Substrate incubation time too long or substrate is degraded	Decrease time exposed to substrate or use a fresh substrate
No signal	No fusion protein expressed	Re-evaluate the expression system and prepare a fresh culture for analysis
	Antibody/Antibody-enzyme conjugate inactive	Use a proven antibody/antibody-enzyme conjugate at appropriate titers
	Degraded substrate	Use a fresh stock of substrate
Low Signal	Low level of fusion protein	Re-evaluate the expression system and prepare a fresh culture for analysis
	Crude cell lysate too dilute	Reduce the lysate dilution factor
	Antibody titer too low	Reduce the dilution of primary and/or secondary antibodies
	Substrate incubation time insufficient	Increase exposure to substrate as long as background levels remain low
High Signal	Crude cell lysate too concentrated	Further dilute crude cell lysate
	Antibody titer too high	Increase the dilution of secondary antibodies
	Substrate incubation time too long	Decrease exposure time to substrate

References

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