INSTRUCTIONS

# Pierce Ig Binding Proteins
## (Protein A, G, A/G and L)

1318.7

| Number | Description |
|---|---|
| **21181** | **Protein A**, 5mg from *Staphylococcus aureus*<br>Molecular Weight: 46,762 (Apparent MW by SDS-PAGE: 42,000)<br>Form: Salt-free powder<br>$A_{280}$ of 0.1% solution: ~0.137 (theoretical)<br>Isoelectric point (pI): 5.16 (theoretical)<br>**Storage:** Upon receipt store at -20°C. Product is shipped with an ice pack. |
| **21184** | **Pierce Recombinant Protein A**, 5mg from *Escherichia coli (E. coli)* |
| **77673** | **Pierce Recombinant Protein A**, 50mg from *E. coli* |
| **77674** | **Pierce Recombinant Protein A**, 500mg from *E. coli*<br>Molecular Weight: ~44,600 (Apparent MW by SDS-PAGE: 45,000)<br>Form: Salt-free powder<br>$A_{275}$ of 0.1% solution: ~0.149<br>Isoelectric point (pI): 4.7-4.8<br>Solubility: ≥ 50mg/mL in water<br>**Storage:** Upon receipt store at -20°C. Product is shipped at ambient temperature. |
| **21193** | **Pierce Recombinant Protein G**, 5mg from *E. coli* |
| **77675** | **Pierce Recombinant Protein G**, 50mg from *E. coli* |
| **77676** | **Pierce Recombinant Protein G**, 500mg from *E. coli*<br>Molecular Weight: ~21,600 (Apparent MW by SDS-PAGE: 32,000)<br>Form: Salt-free powder<br>$A_{280}$ of 0.1% solution: 1.0<br>Isoelectric point (pI): 4.5<br>Solubility: ≥ 50mg/mL in water<br>**Storage:** Upon receipt store at -20°C. Product is shipped at ambient temperature. |
| **21186** | **Pierce Recombinant Protein A/G**, 5mg from *E. coli* |
| **77677** | **Pierce Recombinant Protein A/G**, 50mg from *E. coli* |
| **77678** | **Pierce Recombinant Protein A/G**, 500mg from *E. coli*<br>Molecular Weight: 50,460 (Apparent MW by SDS-PAGE: 40,000-45,000)<br>Form: Salt-free powder<br>$A_{280}$ of 0.1% solution: ~0.505<br>Isoelectric point (pI): 4.65<br>Solubility: ≥ 50mg/mL in water<br>**Storage:** Upon receipt store at -20°C. Product is shipped at ambient temperature. |

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| | |
|---|---|
| **21189** | **Pierce Recombinant Protein L**, 1mg from *E. coli* |
| **77679** | **Pierce Recombinant Protein L**, 50mg from *E. coli* |
| **77680** | **Pierce Recombinant Protein L**, 500mg from *E. coli* |

Molecular Weight: ~35,800 (Apparent MW by SDS-PAGE: 36,000)

Form: Salt-free powder

$A_{280}$ of 0.1% solution: ~0.80

Isoelectric point (pI): 4.8

Solubility: 50mg/mL in Ammonium Bicarbonate buffer

**Storage:** Upon receipt store at 4°C. Product is shipped at ambient temperature.

**Note:** Store reconstituted product for up to one month at 4°C. For long-term storage, aliquot, add an equal volume of glycerol and store at -20°C.

## Introduction

### Protein A

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus* that consists of a single polypeptide chain and contains little or no carbohydrate.[1,2] Recombinant Protein A is produced in *E. coli* and essentially functions the same as native Protein A. The Protein A molecule contains five high-affinity ($K_a = 10^8$/mole) binding sites capable of interacting with the Fc region from IgG of several species including human and rabbit (Table 1). Optimal binding occurs at pH 8.2, although binding is also good at neutral or physiological conditions (pH 7.0-7.6)

The interaction between Protein A and IgG is not equivalent for all species.[4,5] Even within a species, Protein A interacts with some subclasses of IgG and not others. For instance, human $IgG_1$, $IgG_2$ and $IgG_4$ bind strongly, while $IgG_3$ does not bind.[3] There are also many instances in which monoclonal antibodies do not bind to Protein A, especially the majority of rat immunoglobulins and mouse $IgG_1$.[6]

### Protein G

Protein G is a bacterial cell wall protein isolated from group G *Streptococci*.[7-10] DNA sequencing of native Protein G identifies two IgG-binding domains and sites for albumin and cell surface binding.[11-16] The albumin and cell surface binding domains have been eliminated from Recombinant Protein G to reduce nonspecific binding and, therefore, can be used to separate IgG from crude samples. Optimal binding occurs at pH 5, although binding is also good at pH 7.0-7.2.

Because Protein G has greater affinity than Protein A for most mammalian IgGs, it may be used for the purification of mammalian IgGs that do not bind well to Protein A (Table 1).[7,8,9,13] Protein G binds with significantly greater capacity than Protein A to several IgG subclasses such as human $IgG_3$, mouse $IgG_1$ and rat $IgG_{2a}$.[7,8,9,10,13] However, Protein G does not bind to human IgM, IgD and IgA.[9,13,16] Differences in binding characteristics between Protein A and Protein G may be explained by the differing compositions in the IgG-binding sites of each protein. The tertiary structures of these proteins are very similar although their amino acid compositions are significantly different.[13-16]

There are inconsistencies in reported binding properties of IgG to Protein G.[10] Variations in isolation and manufacturing methods for Protein G may affect IgG binding, partially because there are differing numbers of IgG-binding sites on various sources of Protein G.[13,14] Binding studies have been performed using native Protein G and several different recombinant forms.[17] Several assay methods have been used to determine relative affinity, including radiolabeling experiments and ELISA techniques. The differing affinity assays may explain some of the inconsistencies. In addition, there are significant binding differences when different buffers are used. Approximately 44% more IgG from rat serum bound to Protein G when Protein G Binding Buffer was used as compared with 20mM Tris Buffer, pH 7.5.

### Protein A/G

Gene fusion of the Fc-binding domains of Protein A and Protein G has resulted in production of a structural and functional chimeric protein with broader binding than either Protein A or Protein G alone (Table 1).[18] During fusion, the Protein G gene sequence coding for the serum albumin-binding site is eliminated. The product obtained is consistent in quality and yield because the bacterial host is engineered to be deficient in major proteolytic activities. Binding is less pH-dependent than either Protein A or Protein G alone, occurring well at pH 5-8.

The extended Fc-binding properties of Protein A/G make it a popular tool in the investigation and purification of immunoglobulins. Protein A/G binds to all human IgG subclasses, IgA, IgE, IgM and to a lesser extent IgD; however, it does not bind mouse IgA, IgM or murine serum albumin.[18] Protein A/G is an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses without interference from these other serum proteins. Individual subclasses of mouse monoclonals are most likely to have stronger affinity to this chimeric protein than to either Protein A or Protein G.[19]

**Protein L**

Protein L is an immunoglobulin-binding protein that was originally derived from the bacteria *Peptostreptococcus magnus*, but is now produced recombinantly.[20,21] Protein L has the unique ability to bind through kappa light chain interactions without interfering with an antibody's antigen-binding site.[22] This gives Protein L the ability to bind a wider range of Ig classes and subclasses than other antibody-binding proteins (Table 1). Protein L will bind to all classes of Ig (IgG, IgM, IgA, IgE and IgD). Protein L will also bind Single chain variable fragments (scFv) and Fab fragments.[22,23] Protein L binds kappa I, III and IV in human and kappa I on mouse. Furthermore, Protein L has a reported isoelectric point of 4.5.[24] However, an isoelectric point of 4.8 was obtained by our scientists (unpublished results).

## Reconstitution Procedure

Thermo Scientific™ Pierce™ Binding Proteins are supplied as salt-free lyophilized powders. Reconstitute in ultrapure water or a physiological buffer such as 10mm Ammonium Bicarbonate or PBS (Product No. 28372, 0.1M phosphate, 0.15M NaCl, pH 7.2). Store reconstituted product up to one month at 4°C. For long-term storage, aliquot, add an equal volume of glycerol and store at -20°C.

## Example ELISA Procedure for Biotinylated Antigens

Antibody binding proteins are often used to coat microplates to capture antibodies in a direct ELISA. Use these proteins only as capture proteins in ELISAs that use a single antibody (e.g., capture an antibody for a cell surface protein that was biotinylated. Streptavidin-HRP is then used to detect the biotinylated antigen bound in the wells). ELISA protocols that use more than one antibody produce false positives when the secondary antibody binds to the coated capture protein, regardless of the antigen's presence.

**A. Materials required**

- **Coating Buffer:** Carbonate-bicarbonate, pH 9.4, such as Thermo Scientific™ BupH™ Carbonate-Bicarbonate Buffer Packs, 0.2M Carbonate-Bicarbonate (Product No. 28382)
- **Microplate:** Pierce™ 96-Well EIA Plates (Product No. 15041) or 8-Well EIA Strip Plates (Product No. 15031)
- **Wash Buffer:** BupH Phosphate Buffered Saline (PBS) Packs, 0.1M phosphate, 0.15M NaCl; pH 7.2 (Product No. 28372) or BupH Tris Buffered Saline Packs, 0.25M Tris, 0.15M NaCl; pH 7.2 (Product No. 28376), with Tween™-20 Detergent added to a final concentration of 0.05%
- **Blocking Buffer:** Use a proteinaceous solution such as Thermo Scientific™ Blocker™ BSA in PBS (Product No 37525) or in TBS (Product No. 37520) and add Tween-20 Detergent (Product No. 28320) to a final concentration of 0.05%
- **Streptavidin-Horseradish Peroxidase Conjugate:** 1mg (Product No. 21126), 2mg (Product No. 21124) or 5mg (Product No. 21127)

**B. Procedure**

1. Dilute binding protein in Coating Buffer to a final concentration of 0.1-10µg/mL.
2. Add 100µL of the binding protein solution to wells. Cover plate and incubate 1 hour at room temperature (RT) or overnight at 4°C.
3. Empty wells and tap inverted microplate onto paper towels.
4. Wash plate with 200µL of wash buffer. Incubate for 5 minutes on shaking platform or plate shaker. Repeat twice for a total of three washes.
5. Block nonspecific binding sites by adding 300µL of Blocking Buffer. Incubate for 1 hour at RT.
6. Empty wells and tap inverted microplate onto paper towels.

7. Dilute the antibody to an appropriate concentration in PBS or TBS and add 100µL of the antibody solution to the wells. Cover plate and incubate for 1 hour at RT.
8. Wash plate with 200µL of wash buffer. Incubate for 5 minutes on a shaking platform or plate shaker. Repeat twice for a total of three washes.
9. Pipette 100µL of biotinylated antigen-containing sample or standard to wells. Cover plate and incubate for 1 hour at RT.
10. Wash plate with 200µL of wash buffer. Incubate 5 minutes on shaking platform or plate shaker. Repeat twice for a total of three washes.
11. Pipette 100µL of Streptavidin-HRP to wells. Optimization will be required to determine the appropriate concentration of Streptavidin-HRP for each assay. Cover plate and incubate for 1 hour at RT.
12. Wash plate with 200µL of wash buffer that lacks Tween-20 Detergent. Incubate five minutes on shaking platform or plate shaker. Repeat twice for a total of three washes.
13. Add appropriate substrate for the detection of HRP and develop according to manufacturer's instructions.

**Table 1. Binding capabilities for immunoglobulin proteins and Thermo Scientific Pierce Proteins L, A, G and A/G.**

| Species | Antibody Class | Protein A | Protein G | Protein A/G | Protein L* |
|---|---|---|---|---|---|
| Human | Total IgG | S | S | S | S* |
| | $IgG_1$ | S | S | S | S* |
| | $IgG_2$ | S | S | S | S* |
| | $IgG_3$ | W | S | S | S* |
| | $IgG_4$ | S | S | S | S* |
| | IgM | W | NB | W | S* |
| | IgD | NB | NB | NB | S* |
| | IgA | W | NB | W | S* |
| | Fab | W | W | W | S* |
| | scFv | W | NB | W | S* |
| Mouse | Total IgG | S | S | S | S* |
| | IgM | NB | NB | NB | S* |
| | $IgG_1$ | W | M | M | S* |
| | $IgG_{2a}$ | S | S | S | S* |
| | $IgG_{2b}$ | S | S | S | S* |
| | $IgG_3$ | S | S | S | S* |
| Rat | Total IgG | W | M | M | S* |
| | $IgG_1$ | W | M | M | S* |
| | $IgG_{2a}$ | NB | S | S | S* |
| | $IgG_{2b}$ | NB | W | W | S* |
| | $IgG_{2c}$ | S | S | S | S* |
| Cow | Total IgG | W | S | S | NB |
| | IgG1 | W | S | S | NB |
| | IgG2 | S | S | S | NB |
| Goat | Total IgG | W | S | S | NB |
| | IgG1 | W | S | S | NB |
| | IgG2 | S | S | S | NB |
| Sheep | Total IgG | W | S | S | NB |
| | $IgG_1$ | W | S | S | NB |
| | $IgG_2$ | S | S | S | NB |
| Horse | Total IgG | W | S | S | ? |
| | IgG(ab) | W | NB | W | ? |
| | IgG(c) | W | NB | W | ? |
| | IgG(T) | NB | S | S | ? |
| Rabbit | Total IgG | S | S | S | W* |
| Guinea Pig | Total IgG | S | W | S | ? |
| Pig | Total IgG | S | W | S | S* |
| Dog | Total IgG | S | W | S | ? |
| Cat | Total IgG | S | W | S | ? |
| Chicken | Total IgY | NB | NB | NB | NB |

*Binding will only occur if the appropriate kappa light chains are present. The binding affinity only refers to species and subtypes with the correct kappa light chains. Lambda light chains and some kappa light chains will not bind.

**Legend:**
W = weak binding
M = medium binding
S = strong binding
NB = no binding
? = information not available

## Related Thermo Scientific Products

| | |
|---|---|
| **20333** | **Pierce Protein A Agarose**, 5mL |
| **20365** | **Pierce Recombinant Protein A Agarose**, 5mL |
| **20398** | **Pierce Recombinant Protein G Agarose**, 2mL |
| **20421** | **Pierce Recombinant Protein A/G Agarose**, 3mL |
| **20510** | **Pierce Recombinant Protein L Agarose**, 2mL |
| **89978** | **NAb™ Protein A Plus Spin Kit** |
| **89979** | **NAb Protein G Spin Kit** |
| **89980** | **NAb Protein A/G Spin Kit** |
| **89981** | **NAb Protein L Spin Kit** |
| **21007** | **Protein A IgG Binding Buffer**, 3.75L |
| **21011** | **Protein G IgG Binding Buffer**, 3.75L |
| **54200** | **Protein A/G IgG Binding Buffer** |
| **21013** | **Gentle Ag/Ab Elution Buffer, pH 6.6** |
| **21012** | **Gentle Ag/Ab Binding Buffer, pH 8.0** |
| **21009** | **IgG Elution Buffer, pH 2.8** |
| **21028** | **IgG Elution Buffer, pH 2.0** |

### References


1. Bjork, I., *et al.* (1972). Some physiochemical properties of protein A from *Staphylococcus aureus*. *Eur J Biochem* **29(3):**579-84.
2. Sjoquist, J., *et al.* (1972). Protein A isolated from *Staphylococcus aureus* after digestion with lysostaphin. *Eur J Biochem* **29(3):**572-8.
3. Hjelm, H., *et al.* (1975). Immunologically active and structurally similar fragments of protein A from *Staphylococcus aureus*. *Eur J Biochem* **57(2):** 95-403.
4. Kronvall, G. and Williams, R.C. (1969). Differences in anti-protein A activity among IgG subgroups. *J Immunol* **103(4):**828-33.
5. Surolia, A., *et al.* (1982). Protein A: Nature's universal anti-body. *Trends in Biochem Sci* **7:** 74-76.
6. Kronvall, G., *et al.* (1970). Protein A reactivity with mouse immunoglobulins. Structural relationship between some mouse and human immunoglobulins. *J Immunol* **105(5):**1116-23.
7. Akerstrom, B. and Bjorck, L. (1986). A physicochemical study of protein G, a molecule with unique immunoglobulin G-binding properties. *J Biol Chem* **261(22):**10240-7.
8. Akerstrom, B., *et al.* (1985). Protein G: a powerful tool for binding and detection of monoclonal and polyclonal antibodies. *J Immunol* **135(4):**2589-92.
9. Bjorck, L. and Kronvall, G. (1984). Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. *J Immunol* **133(2):**969-74.
10. Eliasson, M., *et al.* (1988). Chimeric IgG-binding receptors engineered from staphylococcal protein A and streptococcal protein G. *J Biol Chem* **263(9):** 4323-7.
11. Akerstrom, B., *et al.* (1987). Definition of IgG- and albumin-binding regions of streptococcal protein G. *J Biol Chem* **262(28):**13388-91.
12. Sjobring, U., *et al.* (1988). Isolation and characterization of a 14-kDa albumin-binding fragment of streptococcal protein G. *J Immunol* **140(5):**1595-9.
13. Fahnestock, S. (1987). Cloned streptococcal protein G genes. *Trends in Biochem Sci* **5:**79-83.
14. Olsson, A., *et al.* (1987). Structure and evolution of the repetitive gene encoding streptococcal protein G. *Eur J Biochem* **168(2):**319-24.
15. Fahnestock, S.R., *et al.* (1986). Gene for an immunoglobulin-binding protein from a group G streptococcus. *J Bacteriol* **167(3):**870-80.
16. Guss, B., *et al.* (1986). Structure of the IgG-binding regions of streptococcal protein G. *EMBO J* **5(7):**1567-75.
17. Faulmann, E.L., *et al.* (1989). Immunological applications of type III Fc binding proteins. Comparison of different sources of protein G. *J Immunol Methods* **123(2):**269-81.
18. Sikkema, W.D. (1989). An Fc-Binding Protein. *Amer Biotech Lab* **7:**42.
19. Eliasson, M., *et al.* (1988). Chimeric IgG-binding receptors engineered from staphylococcal protein A and streptococcal protein G. *J Biol Chem* **263(9):**4323-7.
20. Bjorck, L. (1988). Protein L: A novel bacterial cell wall protein with affinity for Ig L chains. *J Immunol* **140(4):**1194-1197.
21. Kastern, W., *et al.* (1992). Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding domain. *J Biol Chem* **267(18):**12820-5.
22. Nilson, B.H., *et al.* (1993). Purification of antibodies using protein L binding framework structures in the light chain variable domain. *J Immunol Methods* **164(1):**33-40.

23. Bottomley, S.P., *et al.* (1995). Cloning, expression and purification of Ppl-1, a kappa-chain binding protein, based upon protein L from *Peptostreptococcus magnus*. *Bioseparation* **5(6):**359-67.

24. Murphy, J.P., *et al.* (1996). Amplified expression and large-scale purification of protein L. *Bioseparation* **6(2):**107-13.

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