LIFE SCIENCE

Protein A, Protein G, Protein L

Antibody-binding proteins such as protein A, protein G and protein L are bacterial proteins that bind specifically to antibodies. They are mainly used for antibody purification, immunoprecipitation (IP) and immunodetection. Each antibody-binding protein exhibits different affinities to various animal species and antibody subtypes. Therefore, it is important to determine the appropriate antibody-binding protein for your sample of interest. TCI has a wide variety of protein A, protein G and protein L conjugates for you to select from.

Protein A

Protein A is a bacterial cell wall component from *Staphylococcus aureus* that specifically binds to the Fc region of IgG derived from various species including human, rabbit, mouse and cow. Our products consist of a recombinant protein A mutant which allows elution of antibodies under mild conditions (pH 4.0). As there is no change in antibody binding affinity, it can be used in the same way as normal protein A.

Protein A Recombinant, expressed in *Escherichia coli*	5mg/vial [P2366]
Protein A Biotin Conjugate	1mg/vial [P2407]
Protein A HRP Conjugate	0.2mg/vial [P2466]
Protein A Agarose	2mL/vial [P2461]

Purification of human IgG using P2461

Protein A agarose in which protein A is bound to an agarose resin by a covalent coupling method can be used in antibody purification and immunoprecipitation. Antibody purification using protein A agarose usually requires an acidic buffer solution between pH 2.5 and pH 3.0 during elution steps. However, this frequently causes the antibody to undergo acid denaturation, changing its higher-order structure, resulting in antibody aggregation and inactivation. TCI's protein A agarose **[P2461]** uses a genetically modified protein A mutant which allows for the elution of antibodies under mild conditions (pH 4.0), under which most antibodies do not denature, as shown in Figure 1.

Protocol:

1. Fill the column with protein A agarose **[P2461]**, and equilibrate it with binding buffer.
2. Add human IgG.
3. Wash the resin with binding buffer, and then elute antibodies with pH 4.0 and pH 3.0 elution buffers.

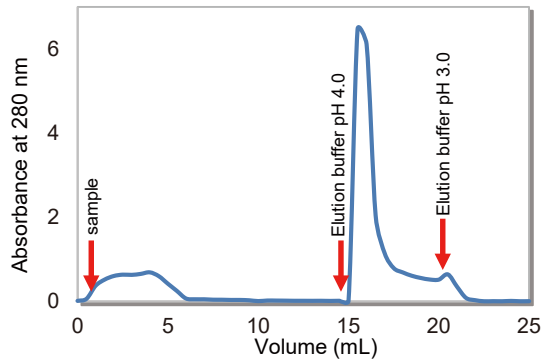


Figure 1. Purification of human IgG using **P2461**

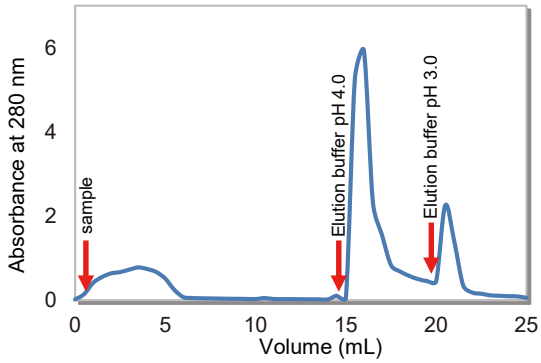


Figure 2. Purification of human IgG using other manufacturer's protein A agarose

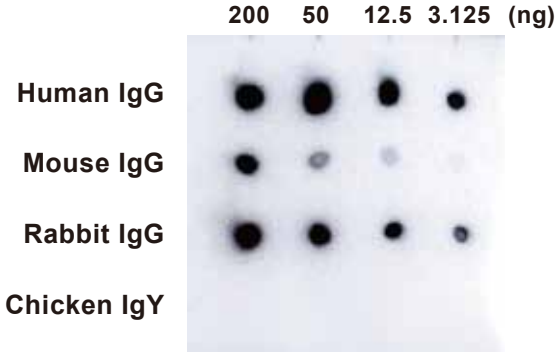
The majority of applied human IgG was successfully eluted at pH 4.0 when using **P2461**.

Protein G

Protein G is a bacterial cell wall component of Group G *Streptococci strain*. It binds specifically to the Fc region of immunoglobulins (especially IgG) and weakly to the Fab fragment.

Protein G Recombinant, expressed in *Escherichia coli*	1mg/vial [P2808]
Protein G Biotin Conjugate	0.2mg/vial [P2959]
Protein G HRP Conjugate	0.2mg/vial [P2962]

Detection of various IgGs by P2962 using the dot-blot method



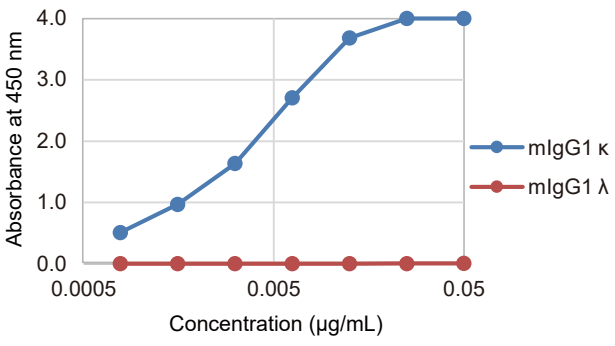
1. Spot Human IgG, mouse IgG, rabbit IgG, and chicken IgY at a 4-fold dilution from 200 ng onto PVDF membrane.
2. Block for 60 minutes at room temperature.
3. Add Protein G HRP Conjugate [P2962] and incubate for 60 minutes at room temperature.
4. Detect the spots by chemiluminescence.

Protein L

Protein L is a cell wall molecule from the bacterial species *Peptostreptococcus magnus*. It binds immunoglobulin light chains in a wide range of species including human, mouse, rat, pig, and hamster, and can bind to any immunoglobulin isoform containing a κ light chain (IgG, IgM, IgA, IgE, and IgD). It can also bind single-chain antibodies (scFv) and Fab fragments with κ light chains.

Protein L Recombinant, expressed in *Escherichia coli*	1mg/vial [P3059]
Protein L Biotin Conjugate	0.2mg/vial [P2998]
Protein L HRP Conjugate	0.2mg/vial [P2999]

Detection of a κ light chain using P2999



1. Coat mouse IgG κ and IgG λ onto ELISA plates.
2. Block for 2 hours at room temperature.
3. Add Protein L HRP Conjugate [P2999] and incubate for 30 minutes at room temperature.
4. Add TMB solution and incubate for 30 minutes.
5. Add 1N HCl solution and measure the absorbance at 450 nm.

Binding Affinity of Protein A, Protein G and Protein L

Animal Species	Antibody Subtype	Protein A	Protein G	Protein L (*)
Human	IgG1	++	++	++
	IgG2	++	++	++
	IgG3	--	++	++
	IgG4	++	++	++
	IgM	-	--	++
	IgD	--	--	++
	IgA	-	--	++
Mouse	IgG1	++	++	++
	IgG2a	++	++	++
	IgG2b	--	++	++
	IgG3	++	++	++
	IgM	-	--	++
Rat	IgG1	-	+	++
	IgG2a	--	++	++
	IgG2b	--	+	++
	IgG2c	++	++	++
Goat	Total IgG	+	++	--
Bovine	Total IgG	+	++	--
Rabbit	Total IgG	++	++	-
Chicken	Total IgY	--	--	--

++: Strong; +: Medium; -: Low; --: None

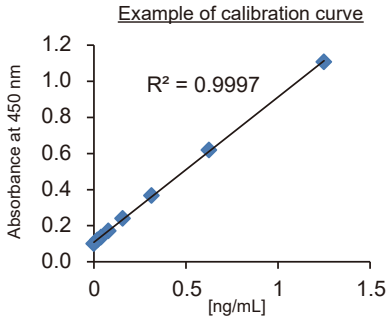
* Protein L binding is restricted to antibodies that contain the right subtypes of κ light chains.

Related Products (Anti-Protein A Antibodies)

Anti-Protein A Chicken Polyclonal Antibody 0.1mg/vial [A3044]
Anti-Protein A Chicken Polyclonal Antibody Biotin Conjugate 0.05mg/vial [A3045]
Anti-Protein A Chicken Polyclonal Antibody HRP Conjugate 0.05mg/vial [A3187]

High-sensitive detection of Protein A by sandwich-ELISA

1. Dilute anti-Protein A antibody [A3044] with sodium carbonate buffer (pH 8.5), and coat on an ELISA plate.
2. Block with 1% BSA / TBS-T for 2 hours.
3. After washing 3 times with TBS-T, add the sample to each well and incubate for 30 minutes.
4. After washing 3 times with TBS-T, add 1 µg/mL of anti-Protein A antibody biotin conjugate [A3045] to each well and incubate for 30 minutes.
5. After washing 3 times with TBS-T, add SA-HRP [S0972] to each well and incubate for 30 minutes.
6. After washing 3 times with TBS-T, add TMB solution and incubate for 30 minutes.
7. Stop the reaction by adding 1 N HCl, and measure the absorbance at 450nm.



Related Products (Peroxidase Substrates)

TMB [for ELISA] (Ready-to-use solution)
(= 3,3',5,5'-Tetramethylbenzidine (Ready-to-use solution))
100mL [T3854]

Application

1. Add 100µL of TMB solution **[T3854]** to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. Add 100µL of 1N HCl solution **[H1202]** to each well to terminate the reaction.
4. Measure the absorbance of each well at 450 nm.

When **T3854** reacts with horseradish peroxidase (HRP), a blue colored soluble reaction product appears thus it can be used for ELISA.
This product cannot be used for Western blotting which needs a precipitate.

Figure.
An example of use by the above method

TMB [for Western blotting] (Ready-to-use solution)
(= 3,3',5,5'-Tetramethylbenzidine (Ready-to-use solution))
100mL [T3855]

AzBTS [for ELISA] (Ready-to-use solution) [for ELISA]
(= 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid Ammonium Salt)
(Ready-to-use solution))
100mL [A3176]

Related Products (Streptavidins)

Streptavidin from *Streptomyces avidinii*	1mg/vial [S0951]
Streptavidin HRP Conjugate	0.1mg/vial [S0972]
Streptavidin FITC Conjugate	0.1mg/vial [S0966]
Streptavidin DTBTA-Eu^{3+} Conjugate	0.1mg/vial [S0993]
Streptavidin R-PE Conjugate	0.1mg/vial [T3885]
Streptavidin Maleimide Conjugate	0.5mg/vial [T3531]

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