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Perfinity Protein A, G or A/G Column

(Product #100-1004, #100-1009, #100-1010)

Reversible adsorption of antibodies that does not interfere with antigen binding

- Binding capacity: 400 ug mouse IgG
- Specially engineered particles enable rapid washing and unprecedented binding kinetics
- A different immunoassay can be performed on each run by changing anti-serum
- Columns can be dedicated to a specific application via antibody cross-linking. Note: This format requires the use of large amounts (400ug) of antibody for optimal binding kinetics

Materials recommended but not provided

- A Perfinity Workstation. A multicolumn system that greatly facilitates automation.
- Perfinity Optimized Wash Solution
- Perfinity Optimized Elution Buffer

I. Operational Specifications –

Recommended Flow Rate	0.5 mL/min
Column Volume	0.100 mL
Pressure (bar)	205 maximum
pH	2-9
Loading Buffer	Perfinity Optimized Wash Solution
Elution Buffer	Perfinity Optimized Elution Buffer
Operating Temperature (°C)	5-40; Ambient (22-25) recommended
Storage Temperature (°C)	4

II. Preparing the column

a. Before you use the column the first time, pump the column with 50 column volumes of Perfinity Optimized Elution Buffer.
b. Equilibrate the column using 50 column volumes of Perfinity Optimized Wash Solution.

III. Preparing and loading the sample

To ensure efficient binding and to prevent column fouling:

a. Heat-treat samples (60°C for 30 minutes) to remove residual fibrinogen that can clog columns on multiple runs.
b. If possible, delipidate samples. Lipids can foul columns.

IV. Sample Loading & Elution -

a. 100ug is the maximum recommended load
b. Concentrate dilute samples - the very high binding constants of the ligand-antibody interaction means that antibody binding is dependant on the mass of IgG in the sample not the concentration. Samples as large as 3mL can be loaded onto the column.
c. To remove most antibodies use an eluent with a pH range of 2-3. Perfinity Optimized Elution Buffer is recommended.
    i. Equilibrate the column with 10 column volumes Perfinity Optimized Wash Solution
    ii. Load sample
    iii. Establish baseline by removing non-specifically bound proteins through an aggressive wash using Perfinity Optimized Wash Solution (e.g. 30 column volumes)
    iv. Elute analyte(s) using Perfinity Optimized Elution Buffer
    v. Re-equilibrate column with 10 column volumes Perfinity Optimized Wash Solution

V. Antibody Cross-linking Protocol (OPTIONAL) –

After loading, incubate column with antibody cross-linking solution (12 mg/mL DSS in 100 mM TEA, 0.25-5 mM DSS in DMF or DMSO, or 0.25-5 mM BS^3 in water or 20 mM sodium phosphate buffer; DSS & BS^3 reactions require quenching with 20-50 mM Tris buffer). Follow incubation with 20 column volume wash with 10 mM TBS pH=7.4.

VI. Cleaning & Maintenance–

Column requires cleanup if any of these indicators appear

- increased bandspreading
- loss of binding capacity
- loss of recovery
- increased pressure drop
- peaks occurring during blank runs

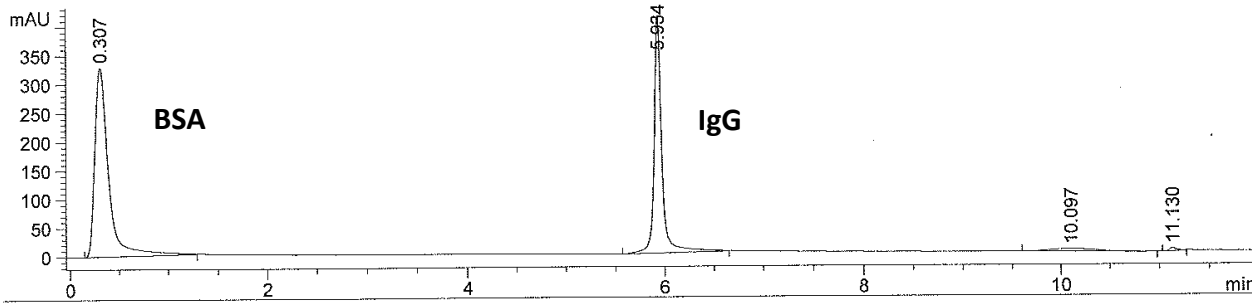
Use 20% (v/v) acetic acid, 0.3 M $MgCl_2$ to regenerate the column. During regeneration, reverse the flow direction and decrease the flow rate in order to flush out any particulates.

VII. Column Validation Protocol –

Perfinity has designed a method to test the performance of the Perfinity A, G, or A/G column. Use this test to troubleshoot column performance whenever in doubt.

1) Prepare solution containing 5 mg/mL BSA and 5 mg/mL IgG in TBS, pH=7.4.
2) Attach Perfinity A, G, or A/G column & perform equilibration procedure listed in step II above.

3) Set-up method to run
- 0-2 min. Perfinity Optimized Wash Solution (loading step – BSA should flow through)
- 2-7 min. Perfinity Optimized Elution Buffer (IgG elution)
- 7-12 min. Perfinity Optimized Wash Solution (column re-equilibration)
*All steps should be performed at 0.500 mL/min.
4) Inject 5 uL of sample and monitor at 280 nm absorbance.
5) Results of method should be similar to chromatogram shown below. Retention times and bandspreading may vary with different HPLC systems, but the general profile should be the same.



VIII. Column Storage –

Store column at 4°C in Perfinity Optimized Wash Solution with end plugs in place to prevent drying. For long term storage, store the column in 0.02% sodium azide in Perfinity Optimized Wash Solution as a preservative.

For additional applications & technical support, please contact Perfinity at (765) 775-1026.

This product is intended for research use only.

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Table 1. Binding capabilities for immunoglobulin proteins and Proteins L, A, B, 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
	IgG_1	W	S	S	NB
	IgG_2	S	S	S	NB
Goat	Total IgG	W	S	S	NB
	IgG_1	W	S	S	NB
	IgG_2	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*
Guingea 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