Ab **SpinTrap**
Ab Buffer Kit

PROTEIN SAMPLE PREPARATION

The Ab SpinTrap™ and the Protein G HP SpinTrap are identical columns. The difference between the article numbers is the pack sizes (50 and 16 columns respectively). Both products include the same protocols: purification of antibodies as well as enrichment of a target protein. For applications in protein enrichment/ immunoprecipitation, please see the *Protein G HP SpinTrap; Protein G HP MultiTrap; Proten A/G Buffer Kit Data file*: 28906790 AB

For simplification, the column is referred to as Ab SpinTrap throughout this document.

Ab SpinTrap are prepacked, single-use spin columns for rapid purification of monoclonal and polyclonal antibodies from serum and cell culture supernatants. The columns are designed for small-scale purification of multiple samples in parallel and are suitable for use in antibody screening experiments (Fig 1). Ab SpinTrap is used with a standard microcentrifuge and one purification run takes less than 20 min. No sample pre-treatment, such as filtration or centrifugation is needed prior to sample application.

The benefits of antibody purification with Ab SpinTrap columns include:

- High purity and yield of antibodies
- Simple and proven method giving reproducible results
- Short purification time due to the prepacked format and no sample pre-treatment

Table 1 lists the main characteristics of Ab SpinTrap.

Ab SpinTrap columns contain Protein G Sepharose™ High Performance medium. The immobilized recombinant protein G is produced in *Escherichia coli* and lacks the albuminbinding region of native protein G.

Fig 1. Ab SpinTrap and Ab Buffer Kit.

Table 1. Characteristics of the Ab SpinTrap and the Ab Buffer kit

Column material	Polypropylene barrel, polyethylene frits
Medium	Protein G Sepharose High Performance
Average bead size	34 µm
Binding capacity[1]	> 1 mg IgG/column
Bed volume	100 µL
Max sample volume	600 µL
Compatibility during use	Stable in all buffers commonly used in antibody purification
pH stability[2] short term (2 h)	2–9
Storage	20 % ethanol
Storage temperature	4°C to 8°C

[1] The binding capacity has been determined with human polyclonal IgG

[2] The ranges estimates based on our experience. Sometimes pH < 3 is required to elute strongly bound IgG species. However at low pH, protein ligands may hydrolyze.

Ab Buffer Kit

Buffer	Content	Formulation	Volume
Binding Buffer	0.2 M Sodium Phosphate, pH 7.0	10×	50 mL
Elution Buffer	1 M Glycine-HCl, pH 2.7	10×	15 mL
Neutralizing Buffer	1 M Tris-HCl, pH 9.0	Ready to use	25 mL

Table 2. The relative binding strengths of antibodies from various species to protein A and protein G as measured in a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined.

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	—
	IgD	—	—
	IgE	—	—
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	—	++++
	IgG_4	++++	++++
	IgM*	variable	—
Avian egg yolk	IgY†	—	—
Cow		++	++++
Dog		++	+
Goat		—	++
Guinea pig	IgG_1	++++	++
	IgG_2	++++	++
Hamster		+	++
Horse		++	++++
Koala		—	+
Llama		—	+
Monkey (rhesus)		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM*	variable	—
Pig		+++	+++
Rabbit		++++	+++
Rat	IgG_1	—	+
	IgG_{2a}	—	++++
	IgG_{2b}	—	++
	IgG_3	+	++
Sheep		+/—	++

* Purified using HiTrap™ IgM Purification HP columns, see related products

† Purified using HiTrap IgY Purification HP columns, see related products

++++ = strong binding

++ = medium binding

— = weak or no binding

Protein G generally has a broader binding specificity than Protein A depending on the origin of the IgG, as shown in Table 2.

Ab SpinTrap can also be used for the preparation of protein samples and enrichment of proteins of interest/immunoprecipitation from clarified cell lysates and biological fluids. Antibodies coupled to the protein G ligand, which is immobilized on the prepacked medium, are used for the capture and enrichment of the protein of interest. The spin columns address the need for flexible, small-scale preparation of protein samples before downstream protein analyses such as gel electrophoresis, liquid chromatography, and LC-MS. IgG immobilizes tightly to the medium and the protein of interest can be enriched separately up to several hundred-fold, depending on the specificity of the antibody.

The key benefits of Ab SpinTrap for protein enrichment are:

- Reproducible capture performance, run for run; required for quantitative and comparative expression studies
- Yield; each protocol includes an Optimization Guide to maximize recovery of your protein of interest
- Optimized for downstream analysis; protocols are designed and tested for several different analytical techniques, for example electrophoresis and LC-MS
- Choice of protocols; a classic protocol for speed or a cross-link protocol ensuring separate elution of the antibody from the protein
- Purity; protocols support performance optimization, modification, and troubleshooting

Operation — antibody purification

The purification of antibodies with Ab SpinTrap can be divided into four stages; equilibration, sample incubation, wash, and elution (Fig 2). Each step involves centrifugation using a microcentrifuge.

Binding of the antibody is performed at neutral pH, and elution by lowering the pH. Eluted material is collected in tubes containing neutralizing buffer to preserve the activity of acid-labile IgG's.

Recommended buffers:

Binding Buffer:	20 mM sodium phosphate, pH 7.0
Elution Buffer:	0.1 M glycine-HCl, pH 2.7
Neutralizing Buffer:	1 M Tris-HCl, pH 9.0

Ab Buffer Kit is available as an accessory for increased convenience (Fig 1). The kit contains stock solutions of Binding, Elution and Neutralizing Buffers optimized for rapid purification of monoclonal and polyclonal IgG with immobilized Protein A or Protein G media. The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible and convenient purification work.

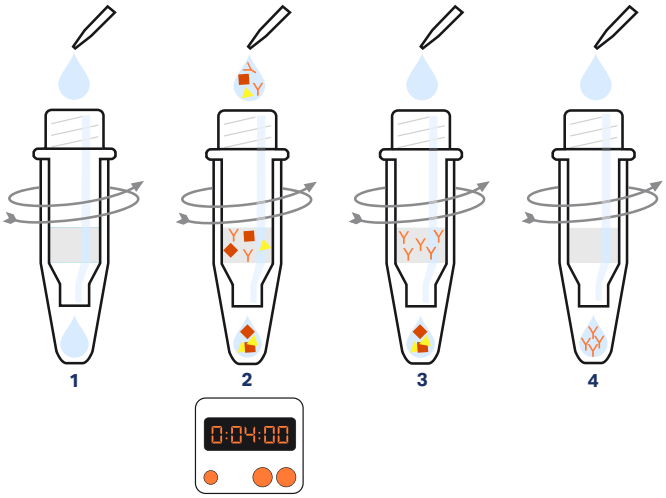


Fig 2. Purifying antibodies with Ab SpinTrap is a simple, four stage procedure that can be performed in less than 20 min using a microcentrifuge: (1) After placing the column in a 2 mL microcentrifuge tube, equilibrate by adding binding buffer and centrifuge; (2) add sample and incubate; (3) wash with binding buffer; (4) elute the target protein with elution buffer.

Maintained recovery with high load of IgG

Ab SpinTrap columns are prepacked with Protein G Sepharose High Performance, which has high protein binding capacity. To demonstrate this feature, different amounts (600 µL sample, 125–2500 µg/column) of human polyclonal IgG (Gammanorm™ = 59% IgG_1, 36% IgG_2, 4.9% IgG_3, 0.5% IgG_4) were loaded on Ab SpinTrap columns.

The amount of eluted IgG was calculated with Lambert- Beers law measuring the absorbance at 280 nm. Results showed that high recoveries (~80%) were obtained up to a load of 1000 µg polyclonal IgG after two elution steps (Fig 3). A small decrease in recovery was seen loading >1000 µg (~70%). Higher recoveries can be obtained with additional elution steps.

Purification of antibodies from cell culture supernatant

Screening or purification of antibodies requires consistent and reproducible results independent of applied antibody concentration. In the previous application, pure IgG was used to show that the recovery, up to a load of 1000 µg (Fig 3), was constant. In this study, experiments with cell culture supernatants were performed to demonstrate the linearity between yield and applied antibody concentration.

Ab SpinTrap columns were tested by purifying mouse monoclonal IgG_{2a} from a cell culture supernatant. The supernatant was diluted 2 and 4 times with binding buffer before loading to columns. Purity and yield of purified antibody were determined by SDS-PAGE (Fig 4) and Lambert-Beers law (measuring the absorbance at 280 nm), respectively. The results show that purity of the eluted IgG_{2a} is very high. Purification with Ab SpinTrap gives reproducible yield and purity at different loading concentrations of antibody (Fig 5).

Multiple load of a cell culture supernatant sample

The concentration of antibodies varies depending on source used in the production. For cell culture, the specific monoclonal antibody concentration is in the range of 0.05–1 mg/mL.

If the monoclonal antibody is present at a low concentration, multiple loading might be needed. The antibody can then be concentrated and eluted in small volumes. Three repeated sample loading (3 × 600 µL) of a cell culture supernatant containing a mouse IgG_{2a} was performed on two Ab SpinTrap columns. The eluted fractions were pooled and analyzed by SDS-PAGE (Fig 6). Results show that the enriched eluted antibody is very pure (> 90%).

Trap product:	Ab SpinTrap
Equilibration:	600 µL Binding buffer
Sample application:	600 µL Gammanorm (125, 500, 1000, 1500, 2000 and 2500 µg IgG/column)
Incubation:	4 min
Wash:	2 × 600 µL Binding buffer
Elution:	2 × 400 µL Elution buffer

Binding, Elution and Neutralizing buffers were prepared from Ab Buffer Kit

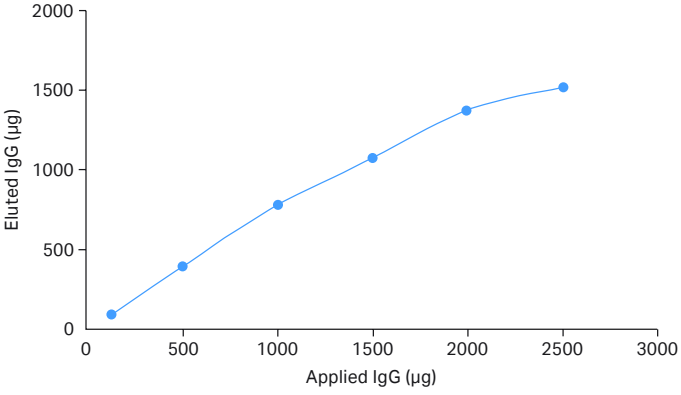
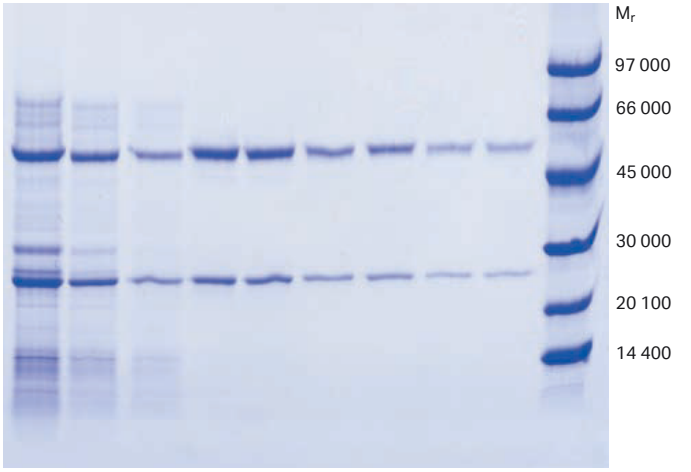


Fig 3. Amount eluted IgG plotted against applied IgG

Trap product:	Ab SpinTrap
Equilibration:	600 µL Binding buffer
Sample application:	600 µL cell culture supernatant (columns 1a, b sample undiluted; columns 2a, b sample diluted × 2; column 3a, b sample diluted × 4. Samples were diluted with Binding buffer)
Incubation:	4 min
Wash:	2 × 600 µL Binding buffer
Elution:	2 × 400 µL Elution buffer

Binding, Elution and Neutralizing buffers were prepared from Ab Buffer Kit



Lane

1. Start material for columns 1a and 1b
2. Start material for columns 2a and 2b
3. Start material for columns 3a and 3b

4-5. Eluted pool, columns 1a and 1b
6-7. Eluted pool, columns 2a and 2b
8-9. Eluted pool, columns 3a and 3b

10. Low molecular weight markers

Fig 4. SDS-PAGE (reducing conditions, ExcelGel™ SDS Gradient 8–18; Coomassie™ staining) of the eluted pools from the purification of different concentrations of mouse monoclonal IgG_{2a} from a cell culture supernatant. Duplicates of three different concentrations were purified.

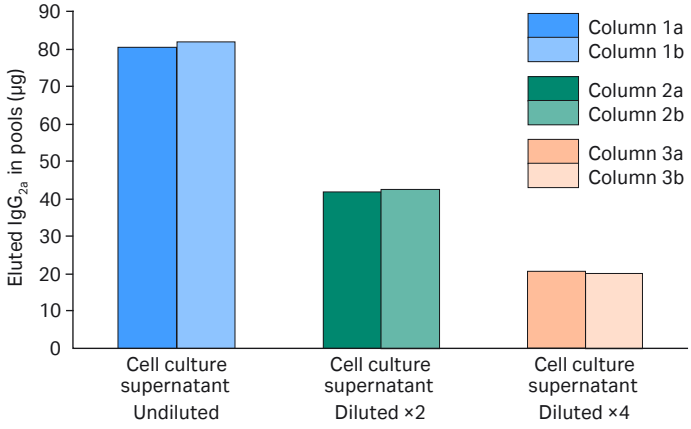


Fig 5. Yield of mouse monoclonal IgG_{2a} in eluted pools. Start material was diluted 2 and 4 times (duplicates) with binding buffer before loading to columns.

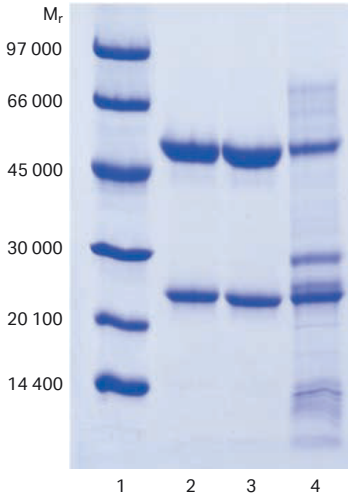
Load of unclarified rabbit anti-HSA serum

Ab SpinTrap can be used for the purification of antibodies from serum without centrifugation, dilution, or filtration of the sample before loading.

The undiluted serum from an immunized rabbit was applied to the column. After washing, the bound antibodies were eluted and purity was checked by SDS-PAGE (Fig 7). The analysis showed that the purity of eluted antibody was > 90%. The amount eluted antibody (calculated with Lambert-Beers law measuring the absorbance at 280 nm) was 2 mg.

Trap product:	Ab SpinTrap
Equilibration:	600 µL Binding buffer
Sample application:	3 × 600 µL cell culture supernatant/column
Incubation:	4 min
Wash:	2 × 600 µL Binding buffer
Elution:	2 × 400 µL Elution buffer

Binding, Elution and Neutralizing buffer were prepared from Ab Buffer Kit



Lane

1. Low molecular weight markers
2. Column 1, eluted pool
3. Column 2, eluted pool
4. Start material

Fig 6. SDS-PAGE (reducing conditions, ExcelGel SDS Gradient 8–18; Coomassie staining) of purified mouse IgG_{2a} from a cell culture supernatant. Three sample applications were performed on the two Ab SpinTrap columns used in the experiment.

Fast purification using prepacked Ab SpinTrap

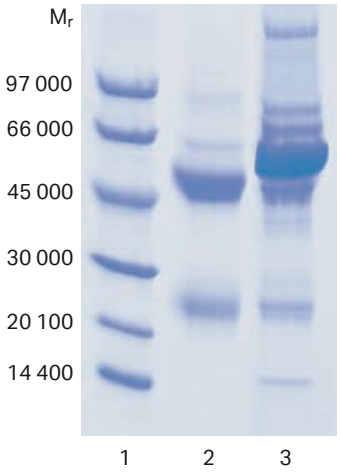
Short and simple purifications are important since they keep time-consuming work and protein degradation to a minimum.

Ab SpinTrap and Proteus Protein G mini column (Prochem) were compared purifying human IgG_1 from a cell culture supernatant. The performance parameters of yield, purity and total purification time (including sample preparation and preparation of columns) were compared.

The total purification time using Ab SpinTrap was less than 20 min compared to 30 min using the Proteus columns. Note that Ab SpinTrap is prepacked and normally no filtration or dilution of sample is needed. Ab SpinTrap showed a slightly higher yield (confirmed by absorbance measurements at 280 nm using Lambert-Beers law) and similar purity (determined by SDS-PAGE, Fig 8) of the purified antibody.

Incubation:	4 min
Wash:	2 × 600 µL Binding buffer
Elution:	2 × 400 µL Elution buffer

Binding, Elution and Neutralizing buffer were prepared from Ab Buffer Kit



Lane

1. Low molecular weight markers
2. Eluted pool (diluted 1:5)
3. Start material (diluted 1:50)

Fig 7. SDS-PAGE (reducing conditions, ExcelGel SDS Gradient 8–18; Coomassie staining) of eluted pool of purified antibody in undiluted serum taken from an immunized rabbit.

Acknowledgements

Human IgG_1 was provided by BioInvent International AB, Lund, Sweden.

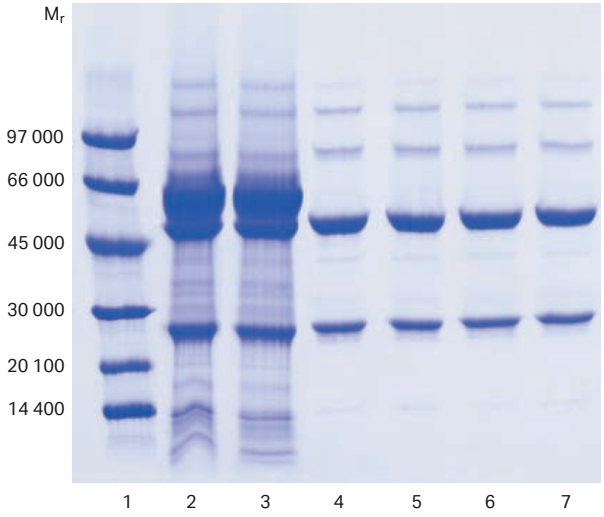
Mouse IgG_{2a} and rabbit anti-HSA serum was provided by Phadia AB, Uppsala, Sweden.

Trap product:	Ab SpinTrap (column 1–2)
Equilibration:	600 µL Binding buffer
Sample application:	600 µL (to be able to compare the results, the sample was diluted 1:2 with Binding buffer and filtered through 0.2 µm)
Incubation:	4 min
Wash:	2 × 600 µL Binding buffer
Elution:	2 × 400 µL Elution buffer

Binding, Elution and Neutralizing buffers were prepared from Ab Buffer Kit

Product:	Proteus Protein G mini (column 3–4)
Equilibration:	2 × 650 µL Binding Buffer
Sample application:	600 µL (diluted 1:2 with Binding buffer and filtered through 0.2 µm, as recommended in protocol)
Wash:	3 × 650 µL Binding buffer
Elution:	2 × 500 µL Elution buffer

Binding, Elution and Neutralizing buffers were obtained from Proteus Protein G Mini Spin Kit



Lane

1. Low molecular weight markers
2. Start material, Ab SpinTrap (diluted 1:2)
3. Start material, Proteus Protein G mini column (diluted 1:2)
4. Ab SpinTrap, column 1, eluted pool
5. Ab SpinTrap, column 2, eluted pool
6. Proteus Protein G mini, column 3, eluted pool
7. Proteus Protein G mini, column 4, eluted pool

Fig 8. SDS-PAGE analysis (reducing conditions, ExcelGel SDS Gradient 8–18; Coomassie staining) of eluted pools from purification of human IgG_1 from a cell culture supernatant comparing Ab SpinTrap and Proteus Protein G mini column (Prochem).

Ordering information

Product	Quantity	Code no.
Ab SpinTrap	50 × 100 µL	28408347
Ab Buffer Kit[1]	1	28903059

[1] Includes: 1 × 50 mL Binding buffer 10× stock solution, 1 × 15 mL Elution buffer 10× stock solution, and 1 × 25 mL Neutralizing buffer.

Related products	Quantity	Code no.
Protein G HP SpinTrap	16 columns	28903134
Protein G HP MultiTrap	4 × 96-well plates	28903135
Protein A HP SpinTrap	16 columns	28903132
Protein A HP MultiTrap	4 × 96-well plates	28903133
Protein A/G HP SpinTrap Buffer Kit	1	28913567
NHS HP SpinTrap	5 mL medium and 24 empty spin columns	28903128
Streptavidin HP SpinTrap	16 columns	28903130
Streptavidin HP MultiTrap	4 × 96-well plates	28903131
Collection plate 500 µL V-bottom (for collection of fractions from MultiTrap)	5 × 96-well plates	28403943

Literature

Related products	Code no.
Antibody purification Handbook	18103746
Affinity chromatography Handbook	18102229
Affinity chromatography columns and media Selection guide	18112186

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