Data Sheet

Protein G SepFast
Protein G SepFast HighRes
Protein G SepFast Large Bead

1. Introduction

Protein G SepFast range is affinity resins with protein G immobilized to highly cross-linked agarose-based SepFast beads.

Protein G binds to the Fc region of IgG from a variety of mammalian species. Protein G SepFast may be used to isolate and purify classes, subclasses and fragments of immunoglobins from any biological fluid or cell culture medium. Protein G SepFast is extremely useful for isolation of immune complexes.

The potential applications of protein G include practically all of the current and projected applications of protein A. Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Compared to protein A, protein G binds more strongly to polyclonal IgG, for example, from cow, sheep and horse. Furthermore, unlike protein A, protein G binds polyclonal rat IgG, human IgG_3 and mouse IgG_1.

The range of Protein G SepFast media is developed and supported for production scale chromatography. Regulatory Support File (RSF) ≥is available to assist process validation and submissions to regulatory authorities.

Protein G SepFast: Suitable for most application from small scale to large bioprocessing scale

Protein G SepFast HighRes: Suitable for processing small quantity of antibodies at reduced time

Protein G SepFast Large Bead: Suitable for processing crude or very viscous feedstocks

2. Product description

Table 1. Characteristics of Protein G SepFast media

Matrix	Highly cross-linked agarose bead
Product format	White to creamy slurry
Binding capacity*	≥ 15 mg human IgG/mL resin
Particle size	50 - 150 μm (Protein G SepFast) 20 - 50 μm (Protein G SepFast HighRes) 150 - 350 μm (Protein G SepFast Large Bead)
Recommended flow rate**	50 to 300 cm/h
Max. operating pressure	0.2 MPa (2 bar, 28 psi)
Chemical stability	Stable in most commonly used buffers, 6 M

	guanidine-HCl; 70% ethanol. 8 M urea, pH 10.5; 0.1 M Glycine-NaOH, pH 11.
Physical stability	Negligible volume variation due to changes in pH or ionic strength.
pH stability	3 to 9 (long term); 2 to 10 (short term)
Sanitization	Sanitize the column with 70% ethanol
Storage temperature	20% ethanol at 2°C to 8°C

*Please note that there might be considerable deviations in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.
**Linear flow rate = volumetric flow rate (cm^3/h)/column cross-sectional area (cm^2)

Table 2. Relative binding strengths for protein A and protein G

Species	**Subclass**	**Protein A binding**[1]	**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	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Llama		-	+
Monkey (rhesus)		++++	++++
Mouse[2]	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit	No distinction	++++	+++
Rat[3]	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_3	+	++
Sheep		+/-	++

[1] ++++ = strong binding; ++ = medium binding; - = weak or no binding
[2] IgG1 from mouse binds more strongly to protein G than to protein A.
[3] Note that IgG from rat binds to protein G coupled to SepFast.

3. Operational conditions

Binding

IgG from most species binds Protein G SepFast at neutral pH and physiological ionic strength.

As a general method we recommend 20mM sodium phosphate, pH 7.0 as binding buffer.

However, the total capacity depends upon several factors, such a the flow rate during sample application, the sample concentration and binding buffer. The table below shows the total capacity under defined conditions for IgG from some species.

Elution

To elute IgG from Protein G SepFast it is necessary to lower the pH to about 3.0 to 2.5 depending on the IgG. As a general method, we recommend 0.1 M glycine buffer, pH 3.0 to 2.5 as elution buffer.

As a safety measure to preserve the activity of acid labile IgG's, it is recommended to add of 60 to 200 µL/mL eluate of 1 M Tris-HCl, pH 9.0, to neutralize the eluted fractions.

Regeneration

After elution, the resin should immediately be washed with 2 to 3 bed volumes of elution buffer followed by re-equilibration with 2 to 3 bed volumes of binding buffer.

In some applications, substances like denatured proteins or lipids, do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures.

4. Cleaning-in-place and Sanitization

Cleaning-in-place (CIP)

Remove strongly bound hydrophobic proteins, lipoproteins and lipids by washing the column with a non-ionic detergent (e.g.Triton™ X-100), 0.1%, at37°C, contact time one minute. Immediately re-equilibrate with at least 5 bed volumes of sterile filtered binding buffer.

Alternatively, wash the column with 70% ethanol and let stand for 12 hours. Re-equilibrate with at least 5 bed volumes of sterile binding buffer.

Sanitization

Sanitization reduces microbial contamination of the chromatography resin to a minimum.

Wash the column with a buffer containing 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours. Re-equilibrate the column with 3 to 5 bed volumes of sterile binding buffer.

Column performance is normally not significantly changed by the cleaning in place or sanitization

procedures described above. These recommended cleaning procedures can be performed directly on the packed column.

5. Immunoprecipitation

Immunoprecipitation is a highly specific and effective technique for analytical separations of target antigens from crude cell lysates.

Getting started

To obtain satisfactory results using immunoprecipitation, all procedures involved must be empirically optimized. For example, selecting cell lysis conditions is very critical and has to be optimized with regard to cell type and how the antigen is to be used. Whereas cells without cell walls (e.g., animal cells) are easily disrupted by treatment with mild detergent, other cells may need some type of mechanical shearing such as sonication or homogenization.

The parameters listed below (lysis buffers, incubation times, volumes, and concentrations) should therefore be regarded as guidelines for initial experiments.

Preparing the resin

Protein G SepFast is supplied preswollen in 20% ethanol. Wash the resin three times with lysis buffer. Centrifuge at 12 000 g for 20 seconds between the washes and discard the supernatant. Prepare a 50% slurry by mixing equal volumes of resin and lysis buffer. Store at 4ºC and mix well before use.

Cell Lysis

Step	Action
1	**Adherent cells**: Remove all culture medium and wash twice with ice-cold PBS. Discard supernatants and drain well. **Cells in suspension**: Collect cells by centrifugation at 1000 g for 5 minutes and discard the culture medium supernatant. Resuspend the pellet in ice-cold PBS, centrifuge and discard the supernatant. Repeat the wash once.
2	**Adherent cells**: Place the tissue culture dish on ice. Add icecold lysis buffer[1] to a concentration of 10^6 to 10^7 cells/mL (1 mL to a cell culture plate, Ø10cm). Incubate on ice for 10 to 15 minutes with occasional rocking. **Cells in suspension**: Suspend the washed pellet in ice-cold lysis buffer[1] at a concentration of 10^6 to 10^7 cells/mL (approx. 10 cell volumes lysis buffer). Incubate on ice for 10 to 15 minutes with gentle mixing.
3	Transfer the cells to a suitable homogenization tube.
4	Further disrupt the cells by sonication, homogenization or passage through a 21

Step	Action
	Gauge needle. Keep the cells on an ice bath to prevent the temperature from rising.
5	Centrifuge at 12 000 g for 10 minutes at 4ºC to remove particular matter.
6	Transfer the lysate (the supernatant) to a fresh tube. Keep on ice.

[1]See section *Buffers and solutions* for help when selecting lysis buffer.

Preclearing (optional)

Antibodies present in the cell lysate may also bind to the resin and thus interfere with subsequent analysis. In such a case preclearing may be desired.

Step	Action
1	Add 50 to 100 uL Protein G SepFast Suspension (50% slurry) to 1 mL cell lysate in a centrifuge tube. Higher volume of resin might be necessary when working with samples due to the large amount of IgG present.
2	Gently mix for 1 hour at 4ºC.
3	Centrifuge at 12 000 g for 20 seconds. Save the supernatant.

Couple antigen to antibody

Step	Action
1	Aliquot samples (500 uL) in new centrifuge tubes.
2	Add: (1) polyclonal serum (0.5 to 5 uL) (2) hybridoma tissue culture supernatant (5 to 100 uL (3) ascites fluid (0.1 to 1 uL), or purified monoclonal or polyclonal antibodies (add the volume corresponding to 1 to 5 ug)

For controls, use non-immune antibodies that are as close to the specific antibody as possible (for example, polyclonal serum should be compared to normal serum from the same species).

Precipitation of the immune complexes

Step	Action
1	Add 50 uL protein G SepFast suspension (50% slurry). Note: it is possible to work with

	volumes down to 10 uL.
2	Gently mix for 1 hour at 4ºC.
3	Centrifuge at 12 000 g for 20 seconds and save the pellet.
4	Wash the pellet three times with 1 mL lysis buffer and once with wash buffer. Centrifuge at 12 000 g for 20 seconds between each wash and discard the supernatants. Note: be very careful when removing the supernatants to avoid loss of beads.

Dissociation and analysis

Step	Action
1	Suspend the final pellet in 30 uL sample buffer
2	Heat to 95 ^{o}C for 3 minutes
3	Centrifuge at 12 000 g for 20 seconds to remove the beads. Carefully remove the supernatant.
4	Add 1 uL 0.1% bromophenol blue
5	Analyze the supernatant by SDS-PAGE, followed by protein staining and/or immunoblotting for detection. Radiolabeled antigens and are detected by autoradiography.

Buffers and solutions

Lysis buffers

Cell lysis must be harsh enough to release the target antigen, but mild enough to maintain its immunoreactivity. Selecting lysing conditions is therefore very critical and has to be individually optimized.

Some commonly used lysis buffers are listed below. NP-40 (IGEPAL CA-630) and RIPA buffer release most soluble cytoplasmic or nuclear proteins without releasing chromosomal DNA and are a good choice of initial experiments. Some parameters that affect the extraction of antigen include salt concentration (0 to 1M), non-ionic detergents (0.1 to 2%), ionic detergents (0.01 to 0.5%) and pH (6 to 9).

Name	Description	Stringency
Low salt	1% IGEPAL CA-630, 50 mM Tris, pH 8.0, 1 mM PMSF	+
Mammalian Protein	Tris-based buffer, 10 mM NaCl, detergent mixture	+

Extraction Buffer	(NP-40, Triton X-100, Tween^{TM}), pH 7.5	
Yeast Protein	Tris-based buffer, 10 mM NaCl, detergent mixture	+
Extraction Buffer	(NP-40, Triton X-100, Tween^{TM}), pH 7.5	
NP-40 (IGEPAL CA-630)	150 mM NaCl, 1% IGEPAL CA-630, 50 mM Tris, pH 8.0, 1 mM PMSF	++
RIPA	150 mM NaCl. 1% IGEPAL CA-630, 0.5% sodium deoxycholate (DOC), 0.1% SDS, 50 mM Tris, pH 8.0, 1 mM PMSF	+++
High salt	500 mM NaCl, 1% IGEPAL CA-630, 50 mM Tris, pH 8.0, 1 mM PMSF	++++

Other buffers/solutions

Name	Description
PBS	1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4
Wash buffer	50 mM Tris, pH 8
Sample buffer(reducing)	1 % SDS, 100 mM DTT, 50 mM, Tris, pH 7.5

6. Storage

For storage, keep the resin at 2°C to 8°C in 20% ethanol. Protein G SepFast must not be frozen.

7. Ordering information

Product	Quantity	Code no.
Protein G SepFast HighRes	5 ml	230701-5ML
	25 ml	230701-25ML
	100 ml	230701-100ML
	250 ml	230701-250ML
	500 ml	230701-500ML
	1 litre	230701-1L
	1 ml HiSep column	230702-1ML
	5 ml HiSep column*	230702-5ML
Protein G SepFast	5 ml	230501-5ML
	25 ml	230501-25ML
	100 ml	230105-100ML
	250 ml	230501-250ML
	500ml	230501-500ML
	1 litre	230501-1L

	1 ml HiSep column	230502-1ML
	5 ml HiSep column*	230502-5ML
Protein G SepFast Large Bead	5 ml	230601-5ML
	25 ml	230601-25ML
	100 ml	230605-100ML
	250 ml	230601-250ML
	500ml	230601-500ML
	1 litre	230601-1L

*Larger quantities available. Contact us for more information

BioToolomics Ltd
Unit 30A, Number 1 Industrial Estate
Consett
County Durham, DH8 6TJ
United Kingdom

www.biotoolomics.com

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