Immunoprecipitation Starter Pack

Immunoprecipitation is a highly specific and effective technique for analytical separations of target antigens from crude cell lysates. In combination with other techniques such as SDS-PAGE and immunoblotting, immunoprecipitation can detect and quantify antigens, determine relative molecular weights, monitor protein turnover and posttranslational modifications, and check for enzyme activity.

nProtein A Sepharose™ 4 Fast Flow and Protein G Sepharose 4 Fast Flow have different binding selectivites, each having a high specificity for the Fc regions of IgG (dependent on species). Together they bind to a wide range of species and, due to their high specificity, provide effective and rapid removal of immune complexes formed between an antigen and its specific antibody in the immunoprecipitation reaction.

Immunoprecipitation Starter Pack contains 2 ml of nProtein A Sepharose 4 Fast Flow, 2 ml of Protein G Sepharose 4 Fast Flow (2 ml equals approximately 80 reactions), and instructions. The instructions describe a generic step-by-step method for immunoprecipitation and include buffer recipes, a troubleshooting guide, and references.

The key benefits of nProtein A Sepharose 4 Fast Flow and Protein G Sepharose 4 Fast Flow are:

- High specificity
- High binding capacity
- Minimal nonspecific adsorption
- Low leakage

Fig 1. Immunoprecipitation Starter Pack consists of 2 ml of nProtein A Sepharose 4 Fast Flow and 2 ml of Protein G Sepharose 4 Fast Flow with instructions for analytical separations of target antigens from crude cell lysates.

Media characteristics

nProtein A is produced in *Staphylococcus aureus* and recombinant protein G is produced in *Escherichia coli*. The proteins are immobilized on Sepharose 4 Fast Flow by the cyanogen bromide method. Sepharose 4 Fast Flow is a stable, highly cross-linked agarose matrix, providing minimal nonspecific adsorption. The resulting media are characterized by high binding capacities and low ligand leakage under a wide range of pH and buffer conditions.

Specificity

Protein A and protein G have different binding selectivities depending on the origin of the IgG. Use Table 1 to select nProtein A Sepharose 4 Fast Flow or Protein G Sepharose 4 Fast Flow according to the source and sub-type of your specific antibody.

Table 1. Relative binding strengths of antibodies from various species to protein A and protein G as measured in a competitive ELISA

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, code no. 17-5110-01

† Purified using HiTrap IgY Purification HP columns, code no. 17-5111-01

++++ = strong binding
++ = medium binding
— = weak or no binding

Applications

Detection of GroEL in E. coli

GroEL (chaperonin 60) belongs to a family of ubiquitous and highly conserved heat-shock molecular chaperone proteins found in prokaryotic and eukaryotic organelles, where they assist in folding of newly imported polypeptides.

In this experiment, immunoprecipitation was used to detect GroEL in crude *E. coli* lysate. The lysate was incubated with antibodies against GroEL. The immune complexes formed were purified using Protein G Sepharose 4 Fast Flow, separated by SDS-PAGE, and analyzed by immunoblotting, see Figure 2.

The experiment demonstrates successful detection of GroEL and the excellent selectivity and minimal nonspecific interactions of Protein G Sepharose 4 Fast Flow.

Platelet-derived growth factor (PDGF) β receptor expression after transfection of porcine aortic endothelial cells

Platelet-derived growth factor (PDGF) is a major growth factor, and overactivity of PDGF is implicated in a number of pathological conditions.

In this experiment, immunoprecipitation was used to investigate if porcine aortic endothelial cells express PDGF-β receptor after transfection.

Parental and transfected cells were left unstimulated or stimulated with PDGF, and lysates were incubated with antibodies against the PDGF-β receptor. The immune complexes were purified using nProtein A Sepharose 4 Fast Flow, separated by SDS-PAGE, and analyzed by immunoblotting using two antibodies, see Figure 3.

The experiment shows that the PDGF-β is correctly autophosphorylated after stimulation with PDGF.

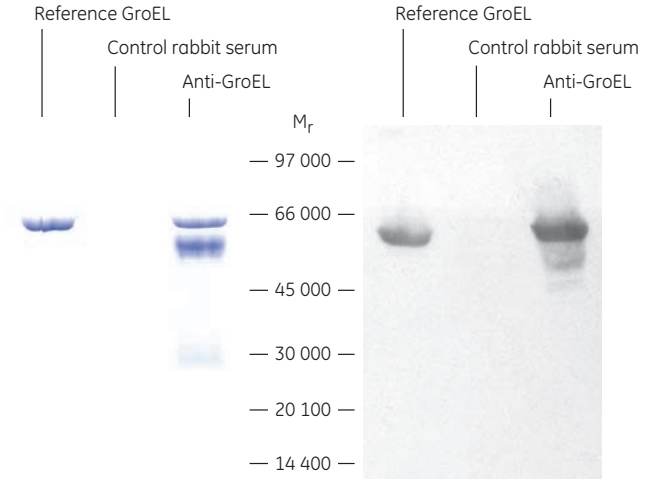
Table 2. Characteristics of Immunoprecipitation Starter Pack

Property	nProtein A Sepharose 4 Fast Flow	Protein G Sepharose 4 Fast Flow
Matrix	Highly cross-linked agarose, 4%	Highly cross-linked agarose, 4%
Ligand	Native protein A	Recombinant protein G lacking albumin-binding region
Ligand density	Approx. 6 mg protein A/ml medium	Approx. 2 mg protein G/ml medium
Ligand coupling method	Cyanogen bromide activation	Cyanogen bromide activation
Binding capacity	> 30 mg human IgG/ml medium	> 20 mg human IgG/ml medium
pH stability[1]	3–9 (long term)	3–9 (long term)

2 ml of medium gives approximately 80 immunoprecipitations (50 µl of 50% slurry for each reaction).

[1] *pH stability, long term*, refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

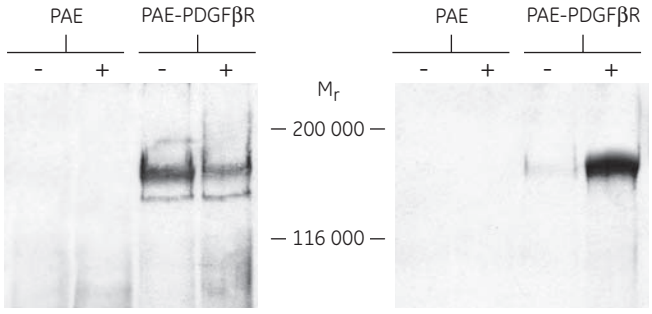
Sample: *E. coli* lysate
Medium: Protein G Sepharose 4 Fast Flow
SDS-PAGE: ExcelGel™ SDS Gradient 8–18
Membrane: Hybond™ ECL™ nitrocellulose membrane
Detection: HRP-conjugated secondary antibodies and ECL reagents



A) SDS-PAGE **B)** Immunoblot with anti-GroEL

Fig 2. Immunoprecipitation of GroEL from *E. coli*. **(A)** SDS-PAGE shows that immunoprecipitation using antibodies against GroEL (anti-GroEL) purifies a protein with a molecular weight (M_r) of 60 000. Proteins were stained with Coomassie™ Brilliant Blue. As SDS-PAGE is an indirect detection procedure, the light and heavy chains of the antibody are also visualized. **(B)** Immunoblot using another antibody against GroEL identifies the M_r 60 000 protein as GroEL.

Sample: Lysates of parental and transfected porcine aortic endothelial cells
Medium: nProtein A Sepharose 4 Fast Flow
SDS-PAGE: ExcelGel SDS Homogeneous 7.5
Membrane: Hybond ECL nitrocellulose membrane
Detection: HRP-conjugated secondary antibodies and ECL reagents



A) Immunoblot with anti-PDGFβR **B)** Immunoblot with anti-P-Tyr

Fig 3. Immunoprecipitation of the PDGF-β receptor from unstimulated (–) and PDGF-stimulated (+) parental (PAE) and transfected (PAE-PDGFβR) porcine aortic endothelial cells. **(A)** Immunoblot using antibodies against the PDGF-β receptor (anti-PDGFbR) demonstrates that the receptor is expressed in the transfected cell line. **(B)** Immunoblot with antibodies against phosphotyrosine (anti-P-Tyr) shows that the PDGF-β receptor is correctly autophosphorylated after stimulation with PDGF.

Acknowledgment

We would like to thank the group of Arne Östman at the Ludwig Institute for Cancer Research, Uppsala, Sweden, for their work with the platelet-derived growth factor β receptor.

References

1. Harlow, E. and Lane, D., *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1999).
2. Bonafacino, J. S., in *Current Protocols in Molecular Biology* **Vol. 2** (Ausubel, F. M., *et al*., eds), John Wiley and Sons, New York, p. 10.18.1 (1991).
3. Springer, T. A., in *Current Protocols in Molecular Biology* **Vol. 2** (Ausubel, F. M., *et al*., eds), John Wiley and Sons, New York, p. 10.16.1 (1991b).
4. *Affinity Chromatography Handbook:* Principles and Methods, GE Healthcare, 18-1022-29.

Ordering information

Product	Quantity	Code no.
Immunoprecipitation Starter Pack	2 × 2 ml	17-6002-35
nProtein A Sepharose 4 Fast Flow, 2 ml Protein G Sepharose 4 Fast Flow, 2 ml		

Related products

nProtein A Sepharose 4 Fast Flow	5 ml	17-5280-01
	25 ml	17-5280-04
Protein G Sepharose 4 Fast Flow	5 ml	17-0618-01
	25 ml	17-0618-02
Protein A Sepharose CL-4B	1.5 g	17-0780-01
Protein A HP SpinTrap™	16 × 100 µl	28-9031-32
Protein G HP SpinTrap	16 × 100 µl	28-9031-34
Protein A HP MultiTrap™	4 × 96-well plates	28-9031-33
Protein G HP MultiTrap	4 × 96-well plates	28-9031-35
ExcelGel SDS Homogeneous 7.5	6 gels	80-1260-01
ExcelGel SDS Gradient 8–18	6 gels	80-1255-53
ExcelGel SDS Buffer Strips	6 + 6 strips	17-1342-01
Hybond ECL (6 × 8 cm)	50 sheets	RPN68D
ECL Western Blotting System	1 kit	RPN2108

Literature	Quantity	Code no.
Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	1	18-1121-86

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