For life science research only.
Not for use in diagnostic procedures.

Protein Agarose

Version: 16
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Affinity chromatography matrix for purification of antibodies and for the immunoprecipitation of proteins.

Cat. No. 11 719 416 001	Protein G Agarose 2 ml
Cat. No. 11 719 408 001	Protein A Agarose 2 ml
Cat. No. 11 243 233 001	Protein G Agarose 5 ml
Cat. No. 11 134 515 001	Protein A Agarose 5 ml
Cat. No. 05 015 952 001	Protein G Agarose 15 ml *Not available in US*
Cat. No. 05 015 979 001	Protein A Agarose 15 ml *Not available in US*

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Cap	Label	Function / Description	Catalog Number	Content
white	Protein A-Agarose	▪ 1 ml settled resin volume of Protein A-Agarose in PBS containing 14 to 19% ethanol. ▪ 2 ml suspension equals 1 ml bed volume.	11 719 408 001	1 bottle, 2 ml
			11 134 515 001	1 bottle, 5 ml
			05 015 979 001	1 bottle, 15 ml
white	Protein G-Agarose	▪ 1 ml settled resin volume of Protein G-Agarose in PBS containing 14 to 19% ethanol. ▪ 2 ml suspension equals 1 ml bed volume.	11 719 416 001	1 bottle, 2 ml
			11 243 233 001	1 bottle, 5 ml
			05 015 952 001	1 bottle, 15 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Cap	Label	Storage
white	Protein A-Agarose	Store at +2 to +8°C. ⚠ ***Do not freeze.***
white	Protein G-Agarose	

1.3. Additional Equipment and Reagent required

For ammonium sulfate precipitation

- Ammonium sulfate, saturated solution (approximately 800 g/l), pH 7.0, precooled at +2 to +8°C
- Starting buffers for Protein A-Agarose and Protein G-Agarose

For purification of Protein A-Agarose

- Starting buffer: 100 mM Tris*-HCl, pH 8.0 (degassed and filtered)
- Washing buffer 1: 100 mM Tris*-HCl, pH 8.0 (degassed and filtered)
- Washing buffer 2: 10 mM Tris*-HCl, pH 8.0 (degassed and filtered)
- Elution buffer: 100 mM glycine, pH 3.0 (degassed and filtered)
- Neutralization buffer: 1.0 M Tris*-HCl, pH 8.0

For purification of Protein G-Agarose

- Starting buffer: 20 mM sodium phosphate, pH 7.0 (degassed and filtered)
- Washing buffer: 20 mM sodium phosphate, 150 mM sodium chloride, 2 mM EDTA, pH 7.0 (degassed and filtered)
- Elution buffer: 100 mM glycine, pH 2.7 (degassed and filtered)
- Neutralization buffer: 1.0 M Tris*-HCl, pH 9.0

For Immunoprecipitation

- Lysis buffer/Washing buffer 1: 50 mM Tris*-HCl, pH 7.5, 150 mM sodium chloride, 1% Nonidet P40*, 0.5% sodium deoxycholate, cOmplete Protease Inhibitor Cocktail Tablets* (50 ml) or cOmplete, Mini* (10 ml), 0.7 µg/ml Pepstatin*
  - *Store in aliquots at −15 to −25°C; mix carefully after thawing.*
- Washing buffer 2 (high salt): 50 mM Tris*-HCl, pH 7.5, 500 mM sodium chloride, 0.1% Nonidet P40*, 0.05% sodium deoxycholate
  - *Stable at +2 to +8°C.*
- Washing buffer 3 (low salt): 50 mM Tris*-HCl, pH 7.5, 0.1% Nonidet P40, 0.05% sodium deoxycholate
  - *Stable at +2 to +8°C.*
- PVDF Membranes*

1.4. Application

Protein A- or Protein G-Agarose affinity matrix can be used:
- To purify antibodies from crude cell extracts.
- For the immunoprecipitation of proteins.

The standard procedures given in this Instructions for Use are general guidelines, but may be modified to suit specific needs.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Protein A- and Protein G-Agarose can be used to purify antibody from:
- Serum
- Culture supernatant
- Ascites
- Cell extracts (adherent, in suspension)
- Hybridoma cells
- Tissues

General Considerations

Use the following guidelines when purifying the text antigens:

Detergents

Always add detergents to the sample lysis buffer to ensure total cell breakage and solubilization of antigen proteins, particularly membrane-associated proteins. In most cases, especially prior to electrophoretic separation, use the detergent recommended in the lysis procedure.

ℹ *Preservation of some antigens requires special solubilization procedures to ensure recovery of a soluble, functionally active protein.*

Inhibitors

Crude extracts contain many proteolytic and other degradative enzymes. To recover undegraded, biologically active antigens, always include inhibitors in all purification buffers. cOmplete Protease Inhibitor Cocktail Tablets inhibit proteases that are generally active at neutral pH in animal tissues, plant tissues, bacteria, yeast, and fungi. If the purification procedure involves lower pHs, use Pepstatin (0.7 µg/ml). To prevent degradation of secondary modifications, such as phosphorylation and glycosylation, use specific enzyme inhibitors. In addition, perform the whole procedure at low temperatures (+2 to +8°C) to help reduce enzymatic degradation.

Wash buffers

Many different buffers may be used to wash Protein A- or Protein G-Agarose-antigen-antibody complexes. The higher the affinity between antibody and antigen, the more stringent the washing conditions can be. The buffers described in this procedure are appropriate for low stringency washes. If the procedure requires higher stringency washes, add 0.5 M sodium chloride or 0.5 M lithium chloride to the first wash buffer. In addition, you may add up to 0.1% SDS to the cell lysis buffer and the first two wash buffers.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Prepare Ig fraction by ammonium sulfate precipitation

For additional information on preparing solutions, see section, **Additional Equipment and Reagent Required**.

Generally, you may use Protein A- and Protein G-Agarose to purify antibody directly from serum, culture supernatant, or ascites. However, to ensure long column life and the maximum possible purification of the antibody, prepare Ig fractions of serum or ascites by ammonium sulfate precipitation as described below; then purify antibody from these fractions.

1. Centrifuge serum, ascites, or culture supernatant for 10 minutes at 500 × *g* to remove cells; save the supernatant.
   – Centrifuge the supernatant for 10 minutes at 10,000 × *g* in a refrigerated centrifuge at +2 to +8°C to remove debris.
   – Carefully remove the layer of fat that may have formed on top of the supernatant.

2. Determine volume of the supernatant (= antibody solution) and transfer it into an appropriate beaker on ice.
   ⚠ *Perform the following steps on ice.*

3. Drop by drop, add an equal volume of saturated Ammonium sulfate solution to the antibody solution while gently stirring the mixture.
   Add the Ammonium sulfate solution slowly to avoid producing a local ammonium sulfate concentration greater than 50% at the site of addition.

4. Gently stir for 1 hour on ice, avoiding the formation of bubbles or froth.

5. Centrifuge for 10 minutes at 10,000 × *g* in a refrigerated centrifuge at +2 to +8°C.
   – Carefully discard the supernatant and resuspend the pellet in Starting buffer.
   The Starting buffer depends on the agarose to be used in the ***Purification*** *sections.*

6. Dialyze against a minimum of three changes of Starting buffer.
   Leave enough space in the dialysis tubing to allow the dialysate to double in volume.

7. Centrifuge the dialysate to pellet any remaining debris.
   – Discard the pellet.

Prepare test antigens and determine antibody concentration

In pilot experiments, use increasing quantities of antibody to precipitate a fixed amount of antigen. Full immunoprecipitation will usually require:

- 0.5 to 5.0 µl of polyclonal antiserum,
- 5 to 100 µl of hybridoma culture supernatant,
- 0.1 to 1.0 µl of ascites fluid, or
- 1 to 5 µg of purified monoclonal or polyclonal antibody.

Purification on Protein A-Agarose

For additional information on preparing solutions, see section, **Additional Equipment and Reagent Required**.

To determine what size column to use, remember that protein A binds 10 to 20 mg of antibody. Serum contains approximately 10 mg/ml total IgG. Tissue culture supernatants contain 20 to 50 µg/ml monoclonal antibody; ascites contain 1 to 10 mg/ml.

1. Pre-equilibrate column with 2 to 5 bed volumes of Starting buffer.

2. To clarify the sample, centrifuge it for 10 minutes at approximately 10,000 × *g* in a refrigerated centrifuge at +2 to +8°C.
   – Discard the pellet.

3. Adjust the pH of the crude antibody sample to 7.5 to 8.0 by adding a volume of 1.0 M Tris, pH 8.0, that is equal to 10% of the initial sample volume.

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4. Slowly pass the sample through the Protein A-Agarose column.

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5. Wash column with approximately 5 to 10 bed volumes of Washing buffer 1.

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6. Wash column with Washing buffer 2 (approximately 5 to 10 bed volumes) until no protein, measured as absorbance at A_{280}, is detected in the effluent.

---

7. Elute bound immunoglobulins with Elution buffer.
   – Collect the eluate in appropriate fractions, such as 500 µl.
   – Fraction collection tubes should already contain a volume of Neutralization buffer equivalent to 20% of the fraction size, for example, 100 µl.
   – Mix the contents of the tube gently to neutralize.
   ⚠ *Keep fractions on ice.*

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8. Identify the immunoglobulin-containing fractions by measuring the absorbance of the fractions at 280 nm (1 A_{280} unit = approximately 0.8 mg Ig protein/ml).
   – Dialyze these purified Ig fractions against PBS.

Purification on Protein G-Agarose

ⓘ *For additional information on preparing solutions, see section,* **Additional Equipment and Reagent Required**.

ⓘ *To determine what size column to use, remember that protein G binds 10 to 20 mg of antibody. Serum contains approximately 10 mg/ml total IgG. Tissue culture supernatants contain 20 to 50 µg/ml monoclonal antibody; ascites contain 1 to 10 mg/ml.*

1. Pre-equilibrate column with 2 to 5 bed volumes of Starting buffer.

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2. To clarify the sample, centrifuge it for 10 minutes at approximately 10,000 × *g* in a refrigerated centrifuge at +2 to +8°C.
   – Discard the pellet.

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3. Adjust the pH of the crude antibody sample to 7.0 by adding Starting buffer.
   ⓘ *If the sample volume is large, for example, a hybridoma culture supernatant, use 1.0 M sodium phosphate, pH 9.0 as the Neutralization buffer.*

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4. Slowly pass the sample through the Protein G-Agarose column.

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5. Wash column with Washing buffer 2 (approximately 5 to 10 bed volumes) until no protein, measured as absorbance at A_{280}, is detected in the effluent.

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6. Elute bound immunoglobulins with Elution buffer.
   – Collect the eluate in appropriate fractions, such as 500 µl.
   – Fraction collection tubes should already contain a volume of Neutralization buffer equivalent to 20% of the fraction size, for example, 100 µl.
   – Mix the contents of the tube gently to neutralize.
   ⚠ *Keep fractions on ice.*

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7. Identify the immunoglobulin-containing fractions by measuring the absorbance of the fractions at 280 nm (1 A_{280} unit = approximately 0.8 mg Ig protein/ml).
   – Dialyze these purified Ig fractions against PBS.

Regeneration of the Protein A- or Protein G-Agarose

1. Wash the columns with several bed volumes of Elution buffer.

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2. Before storing, re-equilibrate columns with Starting buffer containing 20% ethanol.
   – Store columns at +2 to +8°C.

Immunoprecipitation and analysis of antigens

The following procedure uses immunoprecipitation to analyze target antigens from complex mixtures of proteins. A specific antibody concentrates the antigen, then an immunoaffinity column, such as Protein A- or Protein G-Agarose purifies the antigen-antibody complexes for analysis.

Variables that influence the efficiency of solubilization and subsequent immunoprecipitation of proteins include the ionic strength and pH of the lysis buffer, the concentrations and types of detergents used, and the presence of divalent cations or other small molecules.

Cell lysis, sample preparation, and immunoprecipitation

For additional information on preparing solutions, see section, **Additional Equipment and Reagent Required**.

1. Precool PBS wash buffer and Lysis buffer on ice or at +2 to +8°C.
   – Using one of the cell-specific techniques below, wash the sample at least twice with precooled PBS to remove any proteins remaining from the culture medium:

Technique	Steps
Adherent cells	Remove culture supernatant, then add precooled PBS. Swirl the dish, scrape the cells to one side of the dish with a suitable device such as a rubber policeman, then remove PBS. Repeat PBS wash and resuspend washed cells in precooled Lysis buffer at a concentration of approximately 10^6 to 10^7 cells/ml.
Suspension cells	Centrifuge cells at approximately 300 × *g* to pellet. Resuspend in precooled PBS, repeat the centrifugation, then discard the supernatant. Repeat PBS wash and resuspend washed cell pellet in precooled Lysis buffer at a concentration of 10^6 to 10^7 cells/ml.
Solid tissue	Resuspend tissue in precooled PBS, centrifuge the suspension at 300 × *g*, then discard the supernatant. Repeat PBS wash and resuspend washed tissue in precooled Lysis buffer at a concentration of 5 to 20 mg tissue/ml.

You will need 1 to 3 ml sample for one immunoprecipitation. For immunoprecipitation in a microcentrifuge tube, you will only need 1 ml.

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2. Precool a Dounce homogenizer or any other type of microhomogenizer on ice.
   – Transfer the sample to the precooled homogenizer.
   – Use approximately 10 repeated strokes of a type B pestle to homogenize the sample.
   The homogenization procedure can critically affect the functional integrity of the target antigen.

---

3. Use one of the following procedures to clarify the homogenized suspension:
   – Centrifuge the homogenate for 10 minutes at 12,000 × *g* in a refrigerated microcentrifuge at +2 to +8°C.
   – Alternatively, centrifuge the homogenate for 45 minutes at 100,000 × *g* in a refrigerated ultracentrifuge at +2 to +8°C.

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4. Carefully transfer the supernatant to a fresh microcentrifuge tube (optimal volume of 1 ml); discard the pellet.

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5. To reduce background caused by nonspecific adsorption of irrelevant proteins to Protein A- and G-Agarose, add 50 µl of homogeneous protein A/G-Agarose suspension (25 µl bed volume) to the sample (1 to 3 ml) and incubate for at least 3 hours or overnight at +2 to +8°C on a rocking platform.

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6 Pellet agarose beads by gravity sedimentation or by centrifugation for 20 seconds at 12,000 × *g* in a microcentrifuge.
– Transfer supernatant to a fresh microcentrifuge tube.

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7 Add an appropriate amount of the specific antibody as described in section, **Preparation of the fractions**, to the sample and incubate for 1 hour at +2 to +8°C on a rocking platform.

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8 Add 50 µl of homogeneous Protein A- or Protein G-Agarose suspension to the mixture and incubate for at least 3 hours or overnight at +2 to +8°C on a rocking platform.

---

9 Collect agarose-antibody-antigen complexes by gravity sedimentation or by centrifugation for 20 seconds at 12,000 × *g* in a microcentrifuge; discard the supernatant.
– Carefully remove supernatant, resuspend the beads in 1 ml Washing buffer 1 and incubate for 20 minutes at +2 to +8°C on a rocking platform.

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10 Repeat the last step.

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11 Collect complexes as in Step 9; discard the supernatant.
– Resuspend the pellet in 1 ml of Washing buffer 2 and incubate for 20 minutes at +2 to +8°C on a rocking platform.
– Pellet the complexes again and discard the supernatant.

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12 Repeat the last step.

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13 Resuspend the pellet in 1 ml of Washing buffer 3 and incubate for 20 minutes at +2 to +8°C on a rocking platform.
– Pellet the complexes again and discard the supernatant.

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14 Remove the last traces of the final wash from the agarose pellet and from the walls and lid of the microcentrifuge tube.
– Resuspend the agarose pellet in 25 to 75 µl of gel-loading buffer.

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15 Denature proteins by heating the suspension to +100°C for 3 minutes.
– Remove Protein A/G-Agarose by centrifuging the suspension for 20 seconds at 12,000 × *g* in a microcentrifuge at +15 to +25°C.
– Transfer supernatant to a fresh tube.

---

16 Analyze an aliquot of the final supernatant by SDS-polyacrylamide gel electrophoresis.

Gel electrophoresis

To separate the immunoprecipitated proteins, use any type of one- or two-dimensional electrophoresis system that provides sufficient protein resolution. For detailed electrophoresis procedures, see one of the standard text books or manuals available from manufacturers of electrophoresis equipment.

Western blotting

After electrophoresis, blot the gel onto a nitrocellulose or PVDF membrane* using a standard western blot procedure. To avoid damaging or contaminating the membrane, always wear gloves when handling. If the membrane is hydrophobic, such as PVDF, prewet membrane prior to protein transfer as recommended by the manufacturer.

2.3. Parameters

Specificity

Protein A and protein G are bacterial cell wall constituents that have specific binding sites for the Fc parts of certain classes of immunoglobulins from various species.

- Protein A binds to varying degrees, IgM, IgA, IgD, and most subclasses of IgG.
- Protein G binds nearly all subclasses of IgG, but not other classes of immunoglobulins. The recombinant form of protein G lacks the BSA binding domain of natural protein G. Thus, recombinant protein G agarose will not bind serum albumin.

Affinities of protein A/G for various IgG subclasses

Antibody	Protein A	Protein G
Human IgG_1	++++	++++
Human IgG_2	++++	++++
Human IgG_3	-	++++
Human IgG_4	++++	++++
Rat IgG_1	-	+
Rat IgG_{2a}	-	++++
Rat IgG_{2b}	-	++
Rat IgG_{2c}	+	++
Mouse IgG_1	+	++++
Mouse IgG_{2a}	++++	++++
Mouse IgG_{2b}	+++	+++
Mouse IgG_3	++	+++

Affinities of protein A/G for immunoglobulins of various species

Antibody	Protein A	Protein G
Human	++++	++++
Horse	++	++++
Cow	++	++++
Pig	+++	+++
Sheep	+/-	++
Goat	-	++
Rabbit	++++	+++
Chicken	-	+
Hamster	+	++
Guinea pig	++++	++
Rat	+/-	++
Mouse	++	++

3. Additional Information on this Product

3.1. Test Principle

Preparation

Recombinant protein A or protein G was covalently coupled to 6% (Protein A-Agarose) or 4% (Protein G-Agarose) crosslinked agarose at a concentration of 3 mg/ml. The affinity matrix is supplied as a 1:1 suspension of agarose beads in phosphate buffered saline (PBS) that contains 20% ethanol.

3.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
ⓘ *Information Note: Additional information about the current topic or procedure.*	
⚠ **Important Note: Information critical to the success of the current procedure or use of the product.**	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Nonidet P-40 Substitute	50 ml, 5 x 10 ml	11 332 473 001
cOmplete	20 tablets in a glass vial, for 50 ml each	11 697 498 001
	3 x 20 tablets in glass vials, for 50 ml each	11 836 145 001
	20 tablets, for 50 ml each	04 693 116 001
cOmplete, Mini	25 tablets in a glass vial, for 10 ml each	11 836 153 001
	30 tablets, for 10 ml each	04 693 124 001
cOmplete, Mini, EDTA-free	25 tablets in a glass vial, for 10 ml each	11 836 170 001
	30 tablets, for 10 ml each	04 693 159 001
cOmplete, EDTA-free	20 tablets, for 50 ml each	04 693 132 001
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Pepstatin	2 mg	10 253 286 001
	10 mg	11 359 053 001
	50 mg	11 524 488 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
cOmplete, EDTA-free	20 tablets in a glass vial, for 50 ml each	11 873 580 001
	3 x 20 tablets in glass vials, for 50 ml each	05 056 489 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications,
please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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