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

Immunoprecipitation Kits

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For the immunoprecipitation of proteins from cellular extracts with protein A and G agarose. The kits contain all reagents necessary for cell lysis, solubilization, stabilization, and immunopurification of proteins.

Cat. No. 11 719 386 001	Protein G 1 kit 20 reactions
Cat. No. 11 719 394 001	Protein A 1 kit 20 reactions

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Immunoprecipitation Kit (Protein A or G), Core Buffer	250 mM Tris-HCl, pH 7.5, stabilized	1 bottle, 50 ml
2	Immunoprecipitation Kit (Protein A or G), NaCl	1 M NaCl, stabilized	1 bottle, 50 ml
3	Immunoprecipitation Kit (Protein A or G), Detergent mix	10 mM Tris-HCl, 10% Nonidet P-40, and 5% sodium deoxycholate, pH 7.5	1 bottle, 15 ml
4	Immunoprecipitation Kit (Protein A or G), Complete Protease inhibitor cocktail tablets	For complete inhibition of proteases during extractions.	1 bottle, 5 tablets
5	Immunoprecipitation Kit (Protein A), Protein A-Agarose suspension	▪ Ready-to-use suspension. ▪ 2 ml suspension equals 1 ml bed volume.	1 bottle, 2 ml suspension
5	Immunoprecipitation Kit (Protein G), Protein G-Agarose suspension	▪ Ready-to-use suspension. ▪ 2 ml suspension equals 1 ml bed volume.	1 bottle, 2 ml suspension

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	Core Buffer	Store at +2 to +8°C. ⚠ ***Do not freeze.***
2	NaCl	
3	Detergent mix	
4	Complete Protease inhibitor cocktail tablets	
5	Protein A-Agarose suspension	
5	Protein G-Agarose suspension	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Microcentrifuge
- Microfuge tubes
- Homogenizer
- Benchtop centrifuge
- Rocking platform
- Type B pestle
- Cell scraper

For cell lysis and sample preparation

See section, **Working Solution** *for additional information on preparing solutions*

- Lysis buffer/Wash buffer 1
- PBS*

For immunoprecipitation of target protein

See section, **Working Solution** *for additional information on preparing solutions.*

- Lysis buffer/Wash buffer 1
- Washing buffer 2 (high salt)
- Washing buffer 3 (low salt)
- PVDF Membranes*

For gel electrophoresis

- Gel-loading buffer
- SDS-polyacrylamide gel

For western blotting

- Nitrocellulose or PVDF membrane*
- Methanol
- Transfer buffer
- 5% Tween 20* (v/v)

1.4. Application

Use the Immunoprecipitation Kits (Protein A and G) for cell lysis, solubilization, stabilization, and immunoprecipitation of proteins.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

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

General Considerations

Detergents

Cell lysis and solubilization of proteins are crucial steps in immunoprecipitation. All methods should ensure solubilization of the target protein in a form that is immunoreactive, undegraded, and ideally biologically active. Ionic strength and pH of the lysis buffer, type and concentrations of the detergents used, and presence of divalent cations influence the efficiency of solubilization and subsequent immunoprecipitation of protein. Detergents are essential for breaking up the cells and keeping proteins, in particular membrane-associated proteins in a soluble state. In most cases, especially when electrophoresis is the analytical step, the Detergent mix delivered with the kit will be suitable. For some antigens, specifically designed solubilization protocols may be required to obtain the protein in an active state.

Inhibitors

In extracts from animal tissue, mainly serine, cysteine, and metalloproteases are found. In plant extracts, serine and cysteine proteases are dominating. Serine and metalloproteases are typical for bacterial extracts. Complete tablets efficiently inhibit serine, cysteine, and metalloproteases in a broad range.
Complete inhibitor contains EDTA in the typical working concentration. To ensure complete inhibition of the metalloproteases, the extraction buffer should not contain divalent cations, such as Ca^{2+}, Mg^{2+}, or Mn^{2+}. The protease inhibitors in the Complete tablets do not form irreversible complexes with SH groups of proteins. In rare cases, aspartic proteases (acid proteases) can interfere with isolations in animal tissues. These proteases, however exhibit pronounced activities only in the acid pH range. If extractions must be performed at these pH values, or single isolation steps are proceeded at low pH range, inhibit aspartic protease activity by adding Pepstatin. When working with biological material containing considerable amounts of atypical proteases not inhibited by the protease inhibitor cocktail, add specific inhibitors, if available. To protect secondary modifications, such as phosphorylation or glycosylation from degradation, specific inhibitors should be added to the buffers. Keep the temperature during the entire procedure between 0 to +4°C to reduce enzymatic degradation.

Specificity of protease inhibitors

Inhibitor	Specificity	Working Concentration
Complete	Serine-, cysteine-, metalloproteases, calpains	1 tablet/25 – 50 ml
Aprotinin	Serine proteases	0.06 – 2 µg/ml
EDTA	Metalloproteases	0.5 – 5 mM
Pefabloc SC	Serine proteases, such as trypsin and chymotrypsin	0.4 – 4 mM
Pepstatin	Aspartic proteases	1 µM
Leupeptin	Serine and cysteine proteases, such as plasmin, trypsin, papain, cathepsin B	1 – 10 µg/ml

Wash conditions

Different buffers are commonly used to wash protein A/G-antigen-antibody complexes. The tighter the binding between antibody and antigen, the more stringent the washing buffer conditions should be. The washing buffers described are used if low stringency conditions are appropriate. If higher stringency is required, increase salt concentration and ionic strength by using 0.5 M NaCl or 0.5 M LiCl for the first wash. Additionally, SDS to a final concentration of 0.1% may be added during cell lysis and in the first two washes.

Antibody concentration

1 ml of protein A/G-Agarose binds approximately 20 mg of pure IgG, equivalent to approximately 2 ml of serum, 200 ml of supernatant from cultured hybridoma cells, or 1 ml ascites fluid.
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
- 1 to 5 µg of purified monoclonal or polyclonal antibody

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

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.

Working Solution

Lysis buffer/Wash buffer 1

The kit contains reagents for 125 ml of Lysis buffer/Wash buffer 1.

1. Prepare at least a minimal volume of 25 ml, sufficient for 4 immunoprecipitations.
2. Mix 5 ml Core Buffer, 3.75 ml NaCl, 2.5 ml Detergent mix, and 1 Complete Tablet.
3. Add water to a final volume of 25 ml.
4. Store 24 hours at +2 to +8°C, or in aliquots at −15 to −25°C for at least 4 weeks.

⚠ ***Mix thoroughly after thawing.***

Reagent	Volume [ml]	Final conc. [25 ml]
Core Buffer	5	50 mM Tris-HCl, pH 7.5
NaCl	3.75	150 mM NaCl
Detergent mix	2.5	1% Nonidet P-40, 0.5% sodium deoxycholate
Complete Tablet	1 Tablet	1 tablet/25 – 50 ml

Wash buffer 2 (high salt)

The kit contains reagents for 50 ml of Wash buffer 2.
i *2 ml of this buffer is required for 1 immunoprecipitation.*

1. Mix 10 ml Core Buffer, 25 ml NaCl, and 0.5 ml Detergent mix.
2. Add water to a final volume of 50 ml.
3. Store at +2 to +8°C. For longer periods, store in aliquots at −15 to −25°C.

⚠ ***Mix thoroughly after thawing.***

Reagent	Volume [ml]	Final conc. [50 ml]
Core Buffer	10	50 mM Tris-HCl, pH 7.5
NaCl	25	500 mM NaCl
Detergent mix	0.5	0.1% Nonidet P-40, 0.05% sodium deoxycholate

Wash buffer 3 (low salt)

The kit contains reagents for 25 ml of Wash buffer 3.
1 ml of this buffer is required for 1 immunoprecipitation.

1. Mix 1 ml Core Buffer and 0.25 ml Detergent mix.

2. Add water to a final volume of 25 ml.

3. Store at +2 to +8°C. For longer periods, store in aliquots at −15 to −25°C.

⚠ ***Mix thoroughly after thawing.***

Reagent	Volume [ml]	Final conc. [25 ml]
Core Buffer	1	10 mM Tris-HCl, pH 7.5
Detergent mix	0.25	0.1% Nonidet P-40, 0.05% sodium deoxycholate

2.2. Protocols

Overview of protocols

Step [Procedure described in section]	Required Solutions	Volume/assay	Total Volume/20 assays [ml]
Cell lysis and sample preparation Section, Protocols, Cell lysis and sample preparation	Lysis buffer Section, Working Solution, Lysis buffer/Wash buffer 1	1 – 3 ml	20 – 60
Preclearing of the sample Section, Protocols, Preclearing with Protein A/G-Agarose	Protein A/G-Agarose suspension (ready-to-use)	50 µl	1
Immunoprecipitation Section, Protocols, Immunoprecipitation of target protein	Protein A/G-Agarose suspension (ready-to-use)	50 µl	1
Wash, 2 times Section, Protocols, Immunoprecipitation of target protein	Wash buffer 1 Section, Working Solution, Lysis buffer/Wash buffer 1	2 ml	40
Wash, 2 times Section, Protocols, Immunoprecipitation of target protein	Wash buffer 2 Section, Working Solution, Wash buffer 2 (high salt)	2 ml	40
Wash, 1 time Section, Protocols, Immunoprecipitation of target protein	Wash buffer 3 Section, Working Solution, Wash buffer 3 (low salt)	1 ml	20

Cell lysis and sample preparation

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

1. Wash cells/tissue at least twice with ice-cold PBS to remove any remaining serum proteins from the culture medium.
   – For one immunoprecipitation reaction, use a sample volume of 1 to 3 ml. When using a microcentrifuge, a volume of 1 ml is optimal.

Technique	Steps
Adherent cells	Wash cells by adding PBS to the monolayer and dispose of the supernatant. Add Lysis buffer precooled to +2 to +8°C to the chilled, washed cell monolayers to achieve a concentration of 10^6 to 10^7 cells/ml. Scrape the cells to one side of the dish using a suitable device.
Suspension cells	Wash cells with PBS by centrifugation and resuspend the pellet. Remove supernatant after the last wash. Resuspend the cell pellet in Lysis buffer cooled to +2 to +8°C to achieve a concentration of 10^6 to 10^7 cells/ml and transfer to an appropriate homogenizing device.
Solid tissue	Wash tissue by adding PBS and dispose of the supernatant. Add Lysis buffer to the sample to achieve a concentration of 5 to 20 mg tissue/ml.

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.

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

Preclearing with Protein A/G-Agarose

To reduce background caused by nonspecific adsorption of irrelevant cellular proteins to Protein A/G-Agarose, perform a preclearing step.

1. Add 50 µl of homogeneous Protein A/G-Agarose suspension (25 µl bed volume) to 1 to 3 ml sample and incubate at +2 to +8°C for at least 3 hours or overnight on a rocking platform.

2. Pellet beads by gravity sedimentation or centrifugation for 20 seconds at 12,000 × *g* in a microcentrifuge.
   – Transfer supernatants to fresh tubes.

Immunoprecipitation of target protein

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

1. To 1 to 3 ml of sample, add an appropriate amount of the specific antibody as described in section, **General Considerations, Antibody concentration**, to the sample and incubate for 1 hour at +2 to +8°C on a rocking platform.

2. 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.

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

4. Repeat the last step.

5. Collect complexes as in Step 3; discard the supernatant.
   – Resuspend the pellet in 1 ml of Wash buffer 2 and incubate for 20 minutes at +2 to +8°C on a rocking platform.
   – Pellet the beads again and discard the supernatant.

6. Repeat the last step.

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

8. 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.

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.

1. 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.

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

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.***

1. If the membrane is hydrophobic, such as PVDF, prewet membrane prior to protein transfer.
   – Moisten with methanol for a few seconds, then soak with transfer buffer for at least 5 minutes.
   – Nitrocellulose membranes should be briefly soaked in water first and then in transfer buffer for at least 5 minutes.

2. It is essential to thoroughly equilibrate the gel in transfer buffer for 5 to 10 minutes prior to transfer.

3. Blot according to standard protocols.

4. The membrane can be stored dry for several months at +2 to +8°C, but must be rewetted before starting immunodetection.
   – Rewet PVDF membranes in methanol or in 5% Tween 20 (v/v) solution.

2.3. Parameters

Specificity

Protein A and G are cell wall proteins, isolated from a specific bacterial strain, which has specific binding sites for certain classes of immunoglobulins from different species.

- Protein A binds to varying degrees to IgM, IgA, IgD, and most subclasses of IgG.
- Protein G binds nearly all subclasses of IgG, but no other classes of immunoglobulins.

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. Troubleshooting

Observation	Possible cause	Recommendation
No signal appears.	Check sample preparation and immunoprecipitation.	To reduce risk of antigen degradation during sample preparation, include additional specific protease inhibitors.
		Increase the concentration of the primary antibody up to 5 µg/ml.
		For low-affinity antibodies, use wash buffers with lower stringency, for example, 150 mM NaCl, no detergent.
		Check the affinity of the primary antibody to Protein A/G-Agarose, see section, **Specificity**. *If the affinity of the primary antibody turns out to be low, change to an appropriate matrix.*
	Check detection.	Check if protein has been transferred properly to the membrane during blotting. If the transfer was not efficient, especially with high molecular weight proteins, change the transfer conditions (prolong the transfer time, increase current, or change to alternative transfer buffers.
		High molecular weight bands are missing; increase blotting time or change the transfer buffer.
		Check the enzyme activity of the secondary antibody conjugate. ▪ Dot different dilutions of enzyme-conjugate onto a blotting membrane and detect directly. ▪ If no signal appears, use fresh enzyme-conjugate and test in the same way. ▪ If still no signal appears, check the detection reagent.
Signals are week.	Check sample preparation and immunoprecipitation.	To reduce the risk of antigen degradation during sample preparation, include additional specific protease inhibitors.
		Optimize the concentrations of primary antibody.
		Prolong the incubation time with primary antibody to several hours at +2 to +8°C.
		Prolong the incubation time with Protein A/G-Agarose to overnight.
		Shorten the washing times; Use wash buffers with lower stringency, for example, 150 mM NaCl, no detergent.
	Check detection.	Increase the amount of protein applied to the gel.
		Check for efficient blotting.
		Prolong the detection time.
High background present.	Check sample preparation and immunoprecipitation.	Samples with high background may need several rounds of preabsorption to remove all the proteins binding nonspecifically to Protein A/G.
		Increase the washing time of the antibody-Protein A/G-Agarose complex after immunoprecipitation; increase the stringency of washing conditions, see section, **General Consideration, Wash conditions**.
		Increased levels of background signals on the blot might be caused by the nonspecific trapping of proteins during centrifugation of Protein A/G-Agarose/antigen complexes. *This can be avoided by gravity-sedimentation of the complexes instead of centrifugation.*
	Check detection.	Use clean equipment, freshly prepared buffers, and new membranes.
		Dilute the protein concentration in the sample.
		Avoid touching the membranes; use gloves and blunt-ended forceps with non-serrated tips.

Nonspecific bands appear.	Remove all the proteins binding nonspecifically to Protein A/G.	Preclear the sample up to three times with Protein A/G-Agarose prior to the immunoprecipitation steps.
		If the serum immunoglobulins cannot be entirely removed during Protein A/G-Agarose preclearing, use serum-free cell culture conditions prior to cell lysis.
		Alternatively, Protein A/G-Agarose can be preloaded with the desired amount of specific antibody and the remaining Protein A/G binding sites can be blocked with nonspecific control antibodies or serum.

4. Additional Information on this Product

4.1. Test Principle

Immunoprecipitation is a widely used method for the analysis of target antigens in complex mixtures of proteins. The protein of interest can be concentrated and immunoaffinity purified in one step on an analytical scale via a specific antibody. Often immunoprecipitated proteins are functionally fully active and can be further analyzed with respect to enzymatic activity, interactions, modifications, and structure.

5. Supplementary Information

5.1. 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.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001

5.4. Trademarks

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

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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