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

Anti-GFP
from mouse $IgG_1κ$ (clones 7.1 and 13.1)

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Anti-Green Fluorescent Protein
Lyophilized, stabilized

Cat. No. 11 814 460 001 200 µg

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-GFP, Anti-Green Fluorescent Protein	▪ White lyophilizate ▪ Mixture of two monoclonal antibodies.	1 vial, 200 µg

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Anti-GFP	Store at +2 to +8°C.

Reconstitution

1. Add 500 µl double-distilled water to the lyophilizate to a final concentration of 0.4 mg/ml.

2. Rehydrate on ice for 30 minutes prior to use.

3. Aliquot and store at −15 to −25°C.
   Alternatively, store at +2 to +8°C for up to 6 months.

1.3. Additional Equipment and Reagent required

For preparation of lyophilizate

- Double-distilled water

For western blotting

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

- Standard electrophoresis equipment
- Transfer buffer
- Methanol
- Western Blocking Reagent*
- PVDF Western Blotting Membranes*
- PBS*
- Tween 20*
- Anti-mouse IgG (H+L)-POD
- Lumi-Light Western Blotting Substrate*
- Plastic wrap
- Lumi-Film Chemiluminescent Detection Film*

For immunoprecipitation

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

- Lysis buffer
- Eppendorf tubes
- Immunoprecipitation Kit (Protein G)*, or
- Protein G Agarose
- Wash buffer
- Microcentrifuge

1.4. Application

Anti-GFP can be used for several applications:

- Verify the expression of Green Fluorescent Protein (GFP) and GFP fusion proteins by western blot analysis.
- Immunoprecipitation of GFP and GFP fusion proteins.

Anti-GFP recognizes both wild type and mutant forms of GFP.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Solution	Composition/Preparation	For use in...
Anti-GFP working solution	Dilute 10 µl of Anti-GFP* concentrate with 10 ml (1:1,000) of a 1:20 dilution of Western Blocking Reagent* in PBS. *This volume provides sufficient antibody for a 10 cm × 10 cm PVDF membrane*.*	Western blotting
Anti-mouse IgG (H+L)-POD working solution	Prepare 10 ml by diluting anti-mouse IgG (H+L)-POD 1:3,000 containing a 1:20 dilution of Western Blocking Reagent in PBS.	Western blotting
Detection solution	See Instructions for Use of the Lumi-Light Western Blotting Substrate*.	Western blotting
Transfer buffer	10% methanol, 24 mM Tris base*, and 194 mM glycine (prepared with TG buffer, 10x).	Western blotting
Lysis buffer	50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, and protease inhibitors. *Also supplied in the Immunoprecipitation Kit (Protein G).*	Immunoprecipitation
Wash buffer	50 mM Tris-HCl, pH 7.5, 0.25 M NaCl, 0.1% Nonidet P-40, 0.05% deoxycholate.	Immunoprecipitation

2.2. Protocols

Western blotting

See section, **Working Solution** *for additional information on preparing solutions.*
The following method has been developed specifically for the Anti-GFP antibody.
For optimal sensitivity of detection, use Anti-GFP along with PVDF membranes, anti-mouse IgG (H+L)-POD, and the Lumi-Light Western Blotting Substrate*.*

1. Perform electrophoresis according to standard protocols.

2. Wet a PVDF membrane in 100% methanol.
   – Equilibrate the membrane in Transfer buffer.
   – Perform western transfer to the PVDF membrane.

3. Block the membrane using gentle rotation for 1 hour at +15 to +25°C in a 1:10 dilution of Western Blocking Reagent* diluted in phosphate-buffered saline (PBS: 1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.0).

4. Incubate the blocked membrane with the Anti-GFP working solution for 1 hour at +15 to +25°C under gentle rotation.
   10 ml provides sufficient antibody solution volume to cover a 10 cm × 10 cm PVDF membrane.

5. Rinse the membrane with PBS containing 0.1% Tween 20* (PBST).

6. Wash the membrane 2 × 10 minutes with PBST.

7. Add the anti-mouse IgG (H+L)-POD secondary antibody preparation (10 ml) to the blot.
   – Incubate the blot for 1 hour at +15 to +25°C under gentle rotation.

8. Rinse membrane with PBST.

9. Wash 3 × 10 minutes with PBST.

10. Following the protocol described with the Lumi-Light Western Blotting Substrate*, add that reagent set's Detection solution to the membrane.
    – Incubate the membrane for 1 minute.

11. Drain excess Detection solution from the membrane.
    – Wrap the membrane in plastic wrap.

12. Expose the membrane to X-ray film or Lumi-Film* in a film cassette for 60 seconds according to the method provided with the Lumi-Light Western Blotting Substrate.
    Substrate development and X-ray film exposure conditions required to achieve optimal signals may vary for each experiment.

Immunoprecipitation

See section, **Working Solution** *for additional information on preparing solutions.*
Some GFP applications may require the concentration of GFP fusion protein samples by immunoprecipitation. The following method has been developed for use with Anti-GFP antibody.

1. Prepare lysates from cells expressing GFP fusion proteins using the Lysis buffer or an equivalent buffer.

2. Add 0.5 to 1.0 ml of lysates to 1.5 ml Eppendorf tubes.
   – Place the tubes on ice.

3. Add 2 to 10 µg of Anti-GFP (5 to 25 µl of bulk concentrate); mix well.
   – Incubate tubes on ice for 1 hour.
   The optimal amount of anti-GFP may vary for each experimental system.

4. Add 50 µl of well-mixed Protein G Agarose suspension.
   Dispense Protein G Agarose suspension using tips with a wide orifice.

5. Incubate tubes under gentle rotation at +2 to +8°C for 3 to 12 hours.

6. Centrifuge the tubes at 5,000 rpm for 20 seconds in a microcentrifuge to pellet the Protein G Agarose beads bearing the adsorbed immunoprecipitates.

7. Remove the supernatants by carefully aspirating the tube contents using transfer pipettes with fine tips.

8. Wash pellets twice with 1 ml Lysis buffer under rotation for 10 minutes at +2 to +8°C, pelleting the beads and aspirating the supernatants.

9. Wash pellets with 1 ml Wash buffer under rotation for 10 minutes, pelleting the beads and aspirating the supernatants.

10. Spin pellets for 20 seconds at full speed in a microcentrifuge.
    – Remove last traces of the final wash.

⚠ ***If high backgrounds are encountered in western analysis of the immunoprecipitates, include additional wash steps and/or change the salt concentration of the Wash buffer.***

2.3. Parameters

Purity

Both Anti-GFP mouse monoclonal antibodies (clones 7.1 and 13.1) are ≥90% pure as determined by HPLC.

3. Additional Information on this Product

3.1. Test Principle

Green fluorescent protein is a spontaneously fluorescent 27-kDa protein originally isolated from the jellyfish *Aequorea victoria*.

- Molecular cloning of the GFP gene and its subsequent expression in heterologous systems have established GFP as a valuable reporter molecule for *in vivo* visualization of gene expression events in a wide variety of cell types and organisms.
- Since GFP requires no additional substrates or cofactors, GFP's green fluorescence can be easily detected using blue or UV light after expression in either prokaryotic or eukaryotic cells.
- In addition, several mutant forms of GFP with unique spectral properties, such as enhanced fluorescence signal and shifts in excitation and emission spectra, have been reported.
- Anti-GFP is a mixture of two high-affinity mouse monoclonal antibodies that were selected for their excellent performance in detection of GFP and a GFP fusion protein.

Preparation

1. Anti-GFP was obtained by immunizing mice with partially purified recombinant *Aequorea victoria* GFP as immunogen.

2. Spleen cells were then fused with myeloma cells to create a variety of hybridoma clones.

3. Hybridoma supernatants were screened for binding to the immunogen and specifically to highly purified recombinant GFP.

4. Hybridomas secreting monoclonal antibodies specific for GFP were isolated and cloned by limiting dilution.

5. Monoclonal antibodies were further screened for performance in western blot and immunoprecipitation applications using GFP fusion proteins.

6. Anti-GFP antibody clones 7.1 and 13.1 were purified to >95% purity as determined by SDS-PAGE and HPLC analyses, then blended and lyophilized in phosphate-buffered saline in the presence of the protein stabilizer gelatin.

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.	
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.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Western Blocking Reagent, Solution	100 ml, 10 blots, 100 cm^2	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm^2	11 921 681 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
Lumi-Light Western Blotting Substrate	1 kit, 4,000 cm^2 membrane, 400 blots with 10 x 10 cm	12 015 200 001
Immunoprecipitation Kits	Protein G, 1 kit, 20 reactions	11 719 386 001
	Protein A, 1 kit, 20 reactions	11 719 394 001
Protein Agarose	Protein G Agarose, 2 ml	11 719 416 001
	Protein A Agarose, 2 ml	11 719 408 001
	Protein G Agarose, 5 ml	11 243 233 001
	Protein A Agarose, 5 ml	11 134 515 001
	Protein G Agarose, 15 ml, *Not available in US*	05 015 952 001
	Protein A Agarose, 15 ml, *Not available in US*	05 015 979 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
Tris hydrochloride	500 g	10 812 846 001
Nonidet P-40 Substitute	50 ml, 5 x 10 ml	11 332 473 001

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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