

Anti-GFP

Mixture of two mouse monoclonal antibodies (Clones 7.1 and 13.1) to the Green Fluorescent Protein;
Stabilized antibody preparation

Cat. No. 1 814 460 200 µg

Version 1, August 1999

Store at +2 to +8°C

Product Description

Introduction

Green Fluorescent Protein (GFP) is a spontaneously fluorescent 27 kDa protein originally isolated from the jellyfish *Aequorea victoria* (1). The molecular cloning of the GFP gene (2) and its subsequent expression in heterologous systems (3) has 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 (e.g., enhanced fluorescence signal and shifts in excitation and emission spectra) have been reported (4,5).

Anti-GFP is a mixture of two high-affinity mouse monoclonal antibodies that were selected for their superior performance in detection of GFP and a GFP fusion protein. Anti-GFP can be used to verify the expression of GFP and GFP fusion proteins by western blot analysis. Anti-GFP can also be used to immunoprecipitate expressed GFP and GFP fusion proteins. Anti-GFP will recognize wild type, as well as the mutant forms, of GFP.

Preparation

Anti-GFP was obtained by immunizing mice with partially purified recombinant *Aequorea victoria* GFP as immunogen. Spleen cells were then fused with myeloma cells to create a variety of hybridoma clones. Hybridoma supernatants were screened for binding to the immunogen and specifically to highly purified recombinant GFP. Hybridomas secreting monoclonal antibodies specific for GFP were isolated and cloned by limiting dilution. Monoclonal antibodies were further screened for performance in western blot and immunoprecipitation applications using GFP fusion proteins. Anti-GFP antibodies clones 7.1 and 13.1 were purified to >95% purity as determined by SDS-PAGE and HPLC analyses. They were then blended and lyophilized in phosphate-buffered saline in the presence of the protein stabilizer gelatin.

Clone

Anti-GFP is a mixture of two clones (7.1 and 13.1) chosen for their superior performance in western blot and immunoprecipitation applications.

Subtype

Both clones are Mouse IgG_{1κ}.

Stability, storage, and shipping

The preparation is a white lyophilizate containing 200 µg of total Anti-GFP IgG. Lyophilized Anti-GFP is stable through the control date printed on the vial when stored at +2 to +8°C. Anti-GFP is shipped at ambient temperature.

Preparation and storage of stock solution

Prepare an antibody stock solution by dissolving the lyophilized Anti-GFP in 500 µl distilled water (rehydrate for 30 minutes on ice prior to use) to produce an antibody concentration of 0.4 mg/ml. Convenient aliquots of the antibody stock solution can be stored at -15 to -25°C. The reconstituted antibody is stable at +2 to +8°C for up to 6 months.

Analysis

Anti-GFP is tested for functionality and purity relative to a reference standard to confirm the quality of each new reagent preparation.

Western blot

A sample of *E. coli* extract containing a recombinant GFP fusion protein is resolved by SDS-PAGE and transferred to a PVDF membrane. When incubated with the blot membrane at a concentration of 0.4 µg/ml, the Anti-GFP antibody binds specifically to the recombinant GFP fusion protein. The antigen/antibody complex on the membrane is visualized with an Anti-Mouse IgG (H+L)-POD (Cat. No. 1 814 168) using Lumi-Light Western Blotting Substrate (POD) (Cat. No. 2 015 200). In contrast, a negative control *E. coli* extract (without GFP fusion protein) shows no significant nonspecific binding of conjugate under standard conditions.

Immuno-precipitation

Anti-GFP is added to an *E. coli* lysate containing an HA epitope-tagged GFP fusion protein. The Anti-GFP/GFP fusion protein immunocomplex is captured on Protein G-agarose, centrifuged, and washed to remove non-binding materials. The immunoprecipitated sample is analyzed by western blotting using Anti-HA-peroxidase conjugate (Cat. No. 1 667 475) and Lumi-Light Western Blotting Substrate (POD) (Cat. No. 2 015 200) for detection.

Purity

Both Anti-GFP mouse monoclonal antibodies (Clones 7.1 and 13.1) are >95% pure as determined by SDS-PAGE and ion-exchange HPLC analyses.

Applications

Western blot

A major use of the Anti-GFP antibody is in the detection of recombinant GFP fusion proteins by western blot analysis. The following method has been developed specifically for the Anti-GFP antibody. For optimal sensitivity of detection, we recommend that you use the Anti-GFP along with PVDF membranes (Cat. No. 1 722 034), Anti-Mouse IgG (H+L)-POD (Cat. No. 1 814 168) and the Lumi-Light Western Blotting Substrate (POD) (Cat. No. 2 015 200).

- Carry out the electrophoresis according to the usual protocols (6). Wet a PVDF membrane (Cat. No. 1 722 034) in 100% methanol. Equilibrate the membrane in transfer buffer containing 10% methanol, 24 mM Tris base, and 194 mM glycine, pH 8.5 (prepared with Buffers in a Box, Premixed TG Buffer [10X], Cat. No. 1 666 886). Perform western transfer to the PVDF membrane.
- After transfer, block the membrane while gently rotating it for 1 hour at room temperature (RT) in a 1:10 dilution of Western Blocking Reagent (Cat. No. 1 921 678) 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).
- Prepare working-strength Anti-GFP reagent by diluting 10 μl of Anti-GFP stock solution with 10 ml (1:1000) of a 1:20 dilution of Western Blocking Reagent in PBS. Incubate the blocked membrane with working-strength Anti-GFP for 1 hour at RT with gentle rotation.

Ten milliliters provides sufficient antibody solution for a 10 cm x 10 cm PVDF membrane.
- Rinse the membrane with PBS containing 0.1% Tween 20 (PBST). Wash the membrane twice, 10 min per wash, with PBST.
- Prepare 10 ml working-strength Anti-Mouse IgG (H+L)-POD (Cat. No. 1 814 168) by diluting the reagent 1:3,000 a 1:20 dilution of Western Blocking Reagent in PBS. Add this secondary antibody preparation to the blot, and incubate the blot for 1 hour at room temperature with gentle rotation.
- Rinse the membrane with PBST. Wash the membrane three times, 10 min per wash, with PBST.
- Follow the protocol described with Lumi-Light Western Blotting Substrate (Cat. No. 2 015 200), and incubate the membrane in detection solution for 1 minute.
- Drain excess Detection Solution from the membrane, and wrap the membrane in plastic wrap. Expose the membrane to X-ray film (e.g., Lumi-Film Chemiluminescent Detection Film, Cat. No. 1 666 657) in a film cassette for 60 seconds.

Substrate-development and X-ray film-exposure conditions required to achieve optimal signals may vary for each experiment.
- Add 50 μl of well-mixed Protein G Agarose suspension (Cat. No. 1 719 416). Dispense Protein G Agarose suspension using tips with a wide orifice.
- Incubate the tubes with gentle rocking at 4°C for 3–12 hours.
- Pellet the Protein G Agarose beads bearing the adsorbed immunoprecipitates by centrifuging the tubes at 5,000 rpm for 20 sec in a microcentrifuge.
- Remove the supernatants by carefully aspirating the tubes' contents using transfer pipettes with fine tips.
- Wash the pellets twice by rotating the tubes with 1 ml of Lysis Buffer for 10 min at 4°C, pelleting the beads, and aspirating the supernatants.
- Wash the pellets once by rotating the tubes with 1 ml Wash Buffer (50 mM Tris-HCl, pH 7.5, 0.25 M NaCl, 0.1% NP-40, 0.05% deoxycholate) for 10 min, pelleting the beads, and removing the supernatants.
- Spin the pellets for an additional 20 sec at full speed in microcentrifuge, and remove any last traces of the final wash.

The immunoprecipitates are now ready for further analysis.

Note: If high backgrounds are encountered in western analysis of the immunoprecipitates, the wash procedure may have to be modified to include additional washes and/or a change in the salt concentration of the Wash Buffer.

References

- Ward, W.W., Cody, C.W., Hart, R.C. and Cormier, M.J. (1980) *Photochem. Photobiol.* **31**: 611–615.
- Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G. and Cormier, M.J. (1992) *Gene* **111**: 229–233.
- Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. and Prasher, D.C. (1994) *Science* **263**: 802–805.
- Cormack, B.P., Valdivia, R., and Falkow, S. (1996) *Gene* **173**: 33–38.
- Cramer, A., Whitehorn, E.A., Tate, E. and Stemmer, W.P.C. (1996) *Nature Biotechnology* **14**: 315–319.
- Dunn, M.M. (1989) *Advances in Electrophoresis* (Radola, B.J., Dumm, M.J. and Chrambach, A., eds.) VCH Verlagsgesellschaft, Weinheim, Vol. 1.

Immuno-precipitation

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.

- Prepare lysates from cells expressing GFP fusion proteins using the Lysis Buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, and protease inhibitors) supplied in the Immunoprecipitation Kit (Protein G) (Cat. No. 1 719 386) or an equivalent buffer.
- Add 0.5–1.0 ml of lysates to a 1.5 ml eppendorf tubes. Place the tubes on ice.
- Add 2–10 μg of Anti-GFP (5–25 μl of bulk concentrate), mix well, and incubate tubes on ice for 1 hour.

Note: The optimal amount of Anti-GFP added for immunoprecipitation may need to be determined empirically for each experimental system.

Related Products

Product	Cat. No.	Pack Size
rGFP	1 814 524	50 µg
Anti-Mouse IgG (H+L)-POD	1 814 168	1 ml
PVDF Western Blotting Membranes	1 722 034 1 722 026	10 sheets (15 x 15 cm) 1 roll (26.5 x 3.75 cm)
Lumi-Light Western Blotting Substrate	2 015 200	400 ml (4000 cm ² membrane)
Lumi-Light ^{PLUS} Western Blotting Kit	2 015 218	1000 cm ² membrane
BM Teton POD Substrate, precipitating	1 544 845	200 mg (4 ml)
Lumi-Film Chemiluminescent Detection Film	1 666 657 1 666 711	100 films 8 x 10 inches; 20.3 x 25.4 cm) 100 films (13.8 x 16.9 inches; 35 x 43 cm)
Immunoprecipitation Kit (Protein G)	1 719 386	20 reactions
Protein G Agarose	1 719 416 1 243 233	2 ml 5 ml
Buffers in a Box Premixed TG Buffer (10X)	1 666 886	4 L
Reporter Gene Assays		
β-Gal ELISA	1 539 426	1 kit (192 tests)
β-Gal Reporter Gene Assay, chemiluminescent	1 758 241	1 kit (500 assays, MTP format; 250 assays, tube format)
β-Gal Staining Set	1 828 673	1 set (100 tests)
CAT ELISA	1 363 727	1 kit (192 tests)
CAT Staining Set	1 836 358	1 set (100 tests with 3.5 cm dishes)
hGH ELISA	1 585 878	1 kit (192 tests)
Luciferase Reporter Gene Assay, high light intensity	1 669 893 1 814 036	200 assays 1000 assays
Luciferase Reporter Gene Assay, constant light signal	1 897 667	1000 assays
SEAP Reporter Gene Assay, chemiluminescent	1 779 842	1 kit (500 assays, MTP format; 250 assays, tube format)

Lumi-Film and Buffers in a Box are trademarks of a member of the Roche Group.
Tween is a registered trademark of ICI Americas Inc., Wilmington, DE, USA.
Nonidet is a registered trademark of Shell International Petroleum Company Limited, U.K.

This product is sold under license from Columbia University. Rights to use this product are limited to research use only. No other rights are conveyed. Inquiry into the availability of a license to broader rights or the use of this product for commercial purposes should be directed to Columbia Innovation Enterprise, Columbia University, Engineering Terrace - Suite 363, new York, New York 10027.

E-mail Address	Country
argentina.biochem@roche.com	Argentina
Biochem.au@roche.com	Australia
Gerhard.Muehlbauer@roche.com	Austria
biochem.be@roche.com	Belgium
biochem.ca@roche.com	Canada
biochem.cn@roche.com	China
Biochemcy.nicosia@roche.com	Cyprus
Bm-comp@bm-comp.cz	Czech Republic
biochem.fi@oriola.fi	Finland
biochem.fr@roche.com	France
biochemInfo.de@roche.com	Germany
tubanegin@istn.irost.com	Iran
agentek@ibm.net	Israel
it.biochem@roche.com	Italy
bmkkbio@cet.co.jp	Japan
Bmskorea@chollian.net	Korea
biocheminfo.nl@roche.com	Netherlands
biochem.nz@roche.com	New Zealand
medinor@medinor.no	Norway
biochem.pt@roche.com	Portugal
biochem.sg@roche.com	Singapore
south_africa.bioboffin@roche.com	South Africa
biochem.es@roche.com	Spain
biochem.se@roche.com	Sweden
BiochemInfo.CH@roche.com	Switzerland
uk.biochem@roche.com	United Kingdom
biochemts.us@roche.com	USA

Argentina 54 1 951-0023-6, 54 1 952-6081-3, 54 1 954-5555 (FAX) 54 1 952-7589; **Australia** (02) 9899 7999; **Austria** (01) 277 870; **Baltic States** (FAX) 49 621 759 4068; **Belgium** (02) 247 4930; **Brazil** +55 (11) 66 3565; **Canada** (450) 686 7050; (800) 361 2070; **Chile** 0 56 (2) 375 2000 (Central); **China** 86 21 6427 5586; **Czech Republic** (0324) 45 54, 58 71-2; **Denmark** 49 13 82 32; **Egypt** 0020 3619947 (Scientific Office) 0020 2 360 9000 (Distrib. Bagneid); **Finland** (09) 429 2342; **France** 04 76 76 30 86; **Germany** (0621) 759 8568; **Greece** (01) 67 40 238; **Hong Kong** (852) 2485 7596; **India** (22) 4314653; **Indonesia** 62 (21) 2523820, Ext. 755; **Iran** 0098 21 208 2266 (Teb Technology) 0098 21 878 5656 (Tuba Nigen); **Republic of Ireland** (800) 40 90 41; **Israel** 972-3-6 49 31 11; **Italy** 039 247 4109-4181; **Japan** 03 5443 5284; **Kenya** 00254 2 744 677; **Kuwait** 4832600-01, -02, -03; **Malaysia** 60 (03) 755 5039; **Mexico** (5) 227 8967, -61; **Netherlands** (036) 539 4911; **New Zealand** (09) 276 4157; **Nigeria** +234 1 521767; **Norway** 22 07 65 00; **Philippines** (65) 27 27 500; **Poland** (22) 374235; **Portugal** (01) 417 17 17; **Russia** (49) 621 759 8636; **Saudi Arabia** 00966 1 4010333; **Singapore** 65 27 27 500; **South Africa** (011) 886 2400; **South Eastern Europe** (FAX) 49 621 759 4068; **South Korea** (02) 3471-6500; **Spain** (93) 201 4411; **Sweden** (08) 404 8800; **Switzerland** 0 417 99 6161; **Taiwan** (02) 736 7125; **Thailand** 66 (2) 274 0708-13; **United Kingdom** (0800) 521 578; **USA** (800) 428 5433

<http://biochem.roche.com/pack-insert/1814460u.pdf>

pi1814460 • 08/99



Roche Diagnostics Corp.
Roche Molecular Biochemicals
9115 Hague Road
PO Box 50414
Indianapolis, IN 46250-0414