GFP Antiserum

Catalog no. R970-01

Version C
103101
28-0228

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Table of Contents

Overview

Introduction

The polyclonal GFP Antiserum allows detection of the *Aequorea victoria* green fluorescent protein (GFP) (Chalfie *et al.*, 1994; Prasher *et al.*, 1992). The purified polyclonal antiserum recognizes wild-type and mutant GFP, as well as GFP fusion proteins expressed in prokaryotic and eukaryotic cells.

Contents

50 microliters of the rabbit polyclonal GFP Antiserum (1.0-2.0 mg protein/ml) is supplied in phosphate-buffered saline (PBS) and 0.01% sodium azide (added as a preservative). Please refer to the label on the tube for the specific concentration of your lot of antiserum. To obtain Material Safety Data Sheet (MSDS) information about sodium azide, please refer to page 7.

The amount of antiserum provided is sufficient to perform 25 western blots using 10 ml working solution per reaction.

Shipping/Storage

The GFP Antiserum is shipped and should be stored at +4°C. This product is guaranteed for six months from the date of receipt if stored properly.

For long-term storage, aliquot the antiserum and store at -20°C or -80°C. Repeated freezing and thawing of the antiserum is not recommended as it may result in loss of antibody activity.

Description of Antiserum

The GFP Antiserum is a purified rabbit polyclonal antiserum raised against recombinant Xpress™-tagged cycle 3-GFP isolated from *E. coli.* Cycle 3-GFP, the GFP cycle 3 mutant described in Crameri *et al.*, 1996, exhibits a >40-fold increase in fluorescent yield over wild-type GFP, but possesses the same excitation and emission maxima as wild-type GFP.

Specificity of the Antiserum

The GFP Antiserum recognizes wild-type and cycle 3-GFP, as well as GFP fusion proteins expressed in prokaryotic and eukaryotic cells. The GFP Antiserum has been tested in immunoblotting (western) and ELISA procedures.

In western blot experiments, the following amounts of purified recombinant GFP, purified cycle 3-GFP, or whole cell lysates containing expressed recombinant cycle 3-GFP gave a strong signal using chemiluminescence or alkaline phosphate detection reagents:

Source of GFP	Amount
Purified recombinant GFP or cycle 3-GFP	50 ng
Cycle 3-GFP-expressing *E. coli* whole cell lysate	10 µg
Cycle 3-GFP-expressing COS-1 whole cell lysate	20 µg

Continued on next page

Overview, continued

Note

The GFP Antiserum was raised against purified recombinant Xpress™ peptide-tagged cycle 3-GFP. Please note that the GFP Antiserum also recognizes proteins containing the Xpress™ peptide. The Xpress™ peptide is an N-terminal tag that contains a polyhistidine tag, the Xpress™ epitope, and an enterokinase cleavage recognition site. The sequence of the Xpress™ peptide is provided below.

Met-Gly-Gly-Ser-His-His-His-His-His-His-Gly-Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-Arg-Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys-

Applications and Recommended Dilutions

We recommend the following dilutions of the supplied solution of GFP Antiserum for these applications:

- For western blots, dilute 1:5,000 into Tris-Buffered Saline (TBS) containing 0.1% Tween-20 and 1% nonfat dry milk or PBS containing 3% (w/v) bovine serum albumin (BSA) immediately before use.
- For ELISA assays, dilute 1:10,000 in PBS containing 3% (w/v) BSA immediately before use. You may want to perform a dilution series to optimize detection conditions for your protein.

If you use a different buffer for washing and blocking your blots, then dilute as described above with that buffer. You may also use gelatin as a blocking agent.

Important

If you use horseradish peroxidase (HRP)-conjugated secondary antibody, be sure to wash the western blot or microtiter wells thoroughly before adding the color development solution. Azide in the antibody buffer will inhibit horseradish peroxidase. In addition, we use 5% dry milk instead of BSA to prevent high background.

Product Qualification

The GFP Antiserum is qualified by western blot analysis using 100 ng of purified, recombinant GFP. A detectable signal must be obtained within 10 minutes using a 1:5,000 dilution of GFP Antiserum followed by an alkaline phosphatase-conjugated secondary antibody and colorimetric detection.

Additional Products

The GFP Antiserum can be used to detect expression of cycle 3-GFP from the vectors contained in the following kits. Other products are also available. For more information on these and other GFP expression products, contact Technical Service (see page 7).

Item	Application	Catalog no.
CT-GFP Fusion TOPO™ Cloning Kit	Fusing cycle 3-GFP to the C-terminus of your PCR product	K4820-01
NT-GFP Fusion TOPO™ Cloning Kit	Fusing cycle 3-GFP to the N-terminus of your PCR product	K4810-01
pTracer™-SV40 Kit	Expression of cycle 3-GFP and your protein from the same vector	K870-01

Western Blot (Immunoblotting)

Introduction

Many western blot procedures are suitable for use with the GFP Antiserum. We have included a general protocol for your convenience. Other protocols are suitable. The table below outlines the basic steps of a western blot.

Step	Description
1	Run an SDS polyacrylamide gel of the purified protein, partially purified protein, or cell lysate with appropriate controls.
2	Transfer the proteins electrophoretically to a nylon or nitrocellulose membrane.
3	Probe the blot with GFP Antiserum.
4	Incubate the blot with the appropriate secondary antibody for chemiluminescence or colorimetric detection.
5	Generate signal using the appropriate detection reagents.

Solutions Required

We use chemiluminescence or colorimetric reagents to detect binding of the GFP Antiserum to GFP, cycle 3-GFP, or GFP fusion proteins. Other methods can be used to detect your recombinant protein. The following materials and solutions are needed for immunoblotting:

- Phosphate-Buffered Saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \bullet 7H_2O$, 1.4 mM KH_2PO_4, pH 7.3)
- Tris-Buffered Saline (TBS: 20 mM Tris-HCl, 500 mM NaCl, pH 7.5)
- Tris-Buffered Saline + Tween 20 (TBST: TBS plus 0.1% Tween-20, w/v)
- Blocking buffer (PBS + 5% nonfat dry milk, w/v)
- Dilution buffer (TBST + 1% nonfat dry milk, w/v)

Chemiluminescence Reagents (please refer to manufacturer's instructions), **or**
Alkaline Phosphatase Reagents

- Secondary Antibody: Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG (whole molecule) (Sigma A3687) or another Anti-Rabbit IgG
- Enzyme Substrates for alkaline phosphatase: Bromochloroindolyl phosphate (BCIP) and nitro blue tetrazolium (NBT). Prepare 10 ml of a 50 mg/ml stock solution of each substrate. Use 100% dimethylformamide (DMF) to dissolve BCIP and 70% DMF to dissolve NBT.
- Alkaline phosphatase buffer (100 mM diethanolamine, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5)

Note

For your convenience, the WesternBreeze® Chromogenic Kit (Catalog no. WB7105) and the WesternBreeze® Chemiluminescent Kit (Catalog no. WB7106) are available from Invitrogen to facilitate secondary detection of the GFP Antiserum by colorimetric or chemiluminescence methods, respectively. For more information, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 7).

Continued on next page

Western Blot, continued

Immunoblotting Protocol

Prepare an SDS polyacrylamide gel (either Tris/Glycine or Tris/Tricine) designed to resolve your recombinant protein. Prepare your samples for electrophoresis. (For information about SDS-polyacrylamide gel electrophoresis, please see Ausubel, *et al.*, 1994.) **Note:** Pre-cast polyacrylamide gels (e.g. NuPAGE® or Novex® Tris-Glycine gels available from Invitrogen) are also suitable. Follow the manufacturer's instructions for use.

Remember that you need to load at least 50 ng of your purified recombinant protein onto the gel in order to get a strong signal.

1. Load your samples and electrophorese your SDS polyacrylamide gel.
2. Transfer proteins to nitrocellulose electrophoretically. Transfer Buffer: 25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3.
3. Run at 100V, 150 mA (100V, 240 mA at the finish) for 1 hour. Be sure to have a cooling system in place. You may also transfer overnight at 30V, 40 mA (which will be 30V, 90 mA at the finish).
4. Remove nitrocellulose and incubate it in 10 ml blocking solution. Gently agitate using a rocker platform for 1 hour at room temperature.
5. Wash nitrocellulose in 20 ml TBST for 5 minutes with gentle agitation.
6. Transfer membrane to a tray containing GFP Antiserum diluted 1:5,000 in 10 ml dilution buffer (2 µl of GFP Antiserum diluted into 10 ml dilution buffer). Incubate with gentle agitation for at least 2 hours.
7. Transfer membrane to a tray containing 20 ml TBST and wash for 5 minutes with gentle agitation.
8. Transfer membrane to a tray containing the alkaline phosphatase-conjugated goat anti-rabbit IgG. Dilute the secondary antibody according to the manufacturer's recommendation into dilution buffer. Incubate with gentle agitation for at least 1 hour.
9. Wash membrane in 20 ml TBST for 10 minutes with gentle agitation. Repeat wash twice more.

Detection Method

If you are using a chemiluminescence kit, please refer to the manufacturer's instructions.

If you wish to use alkaline phosphatase to detect your protein, the protocol on the next page is provided for your convenience. Other detection systems may be used. Please refer to Harlow and Lane, 1988 for a discussion of these systems.

Continued on next page

Western Blot, continued

Alkaline Phosphatase Detection Reaction

1. Transfer membrane from Step 9, previous page, to a tray containing TBS and wash for 5 minutes to remove detergent.
2. Prepare fresh substrate solution immediately before use.

   For alkaline phosphatase-conjugated antibody, add 66 µl of the NBT stock to 10 ml alkaline phosphatase buffer and mix well. Then add 33 µl of the BCIP stock and mix thoroughly. Use within 1 hour.
3. Rinse the blot twice with alkaline phosphatase buffer, then add 10 ml of the substrate solution. Incubate with gentle agitation at room temperature and watch for color development. Proceed to the next step

   When detecting higher concentrations of protein, the purple signal should develop within 10 minutes. Lower concentrations will take longer to develop but should be visible within 30 minutes. Color development will continue for up to 4 hours; however, high backgrounds will occur with longer incubation times.
4. Stop the color development by washing the membrane in distilled water for 10 minutes. Change the water at least once during the 10 minute incubation.
5. Air-dry membrane on filter paper.

Troubleshooting

Troubleshooting

The table below lists some potential problems and possible solutions that you may use to help you troubleshoot your western blotting.

Problem	Reason	Solution
No signal	Poor or no transfer	Stain membrane with Ponceau S to check degree of transfer, then re-run the gel and repeat transfer
	Antibody too dilute	Use more antibody
	Protein too dilute	Load more protein
	Old detection reagents	Prepare fresh detection reagents immediately before use
High background	Antibody too concentrated	Titrate the antibody and use the maximal dilution that gives a detectable signal in a reasonable amount of time
	Insufficient blocking	Increase incubation time in blocking solution Include Tween-20, BSA, or other blocking agents in the blocking and washing solutions
Multiple protein bands	Proteolysis of the protein	Use protease inhibitors when preparing cell lysates
	Inefficient reduction of the protein	Resuspend samples in SDS-PAGE sample buffer containing fresh reducing agent and boil the samples for 5 minutes prior to electrophoresing your gel

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

United States Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, please visit our Web site (www.invitrogen.com) and follow the instructions below.

1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

Continued on next page

Technical Service, continued

Emergency Information

In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company
Voice: 1-760-602-8700

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification for Cycle 3 GFP

The 'cycle 3' mutant GFP was produced by Maxygen, Inc. using DNA shuffling technology. Commercial licensing inquiries should be directed to:

Affymax Research Institute
4001 Miranda Avenue
Palo Alto, CA 94304

References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1994) *Current Protocols in Molecular Biology.* Greene Publishing Associates and Wiley-Interscience, New York.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W., and Prasher, D. C. (1994). Green Fluorescent Protein as a Marker for Gene Expression. Science *263*, 802-805.

Crameri, A., Whitehorn, E. A., Tate, E., and Stemmer, W. P. C. (1996). Improved Green Fluorescent Protein by Molecular Evolution Using DNA Shuffling. Nature Biotechnology *14*, 315-319.

Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.

For Immunoprecipitation, see Chapter 11, pp. 421-470.

For Immunoblotting (westerns), see Chapter 12, pp. 471-510.

For Immunoassays (ELISA), see Chapter 14, pp. 553-612.

Prasher, D. C., Eckenrode, V. K., Ward, W. W., Pendergast, F. G., and Cormier, M. J. (1992). Primary Structure of the *Aequorea Victoria* Green-Fluorescent Protein. Gene *111*, 229-233.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press. Plainview, New York.