

Anti-GFP Antibodies

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Anti-GFP, rabbit polyclonal serum (A6455)	100 μL, with 0.01% thimerosal	Not applicable	• 2–6°C • Protect from light	When stored undiluted as directed, products are stable for at least 3 months.
Anti-GFP, rabbit IgG fraction (A11122)	100 μL	2 mg/mL solution in PBS, pH 7.2, 5 mM azide		For longer storage, aliquot the solution into single-use aliquots and freeze at ≤–20°C. Frozen aliquots are stable for at least 6 months.
Anti-GFP, chicken IgY fraction (A10262)				
Anti-GFP mouse monoclonals 3E6 (A11120, isotype IgG _{2a}) and 11E5 (A11121, isotype IgG ₁) [†]	100 μg	Not applicable	• ≤–20°C • Desiccate • Protect from light	When stored dry as directed, products are stable for at least 6 months.
GFP, ABfinity™ recombinant rabbit monoclonal antibody - unconjugated (anti-GFP, rabbit mAb) (G10362)				
†These antibodies have been purified from mouse hybridoma supernatants by protein G chromatography and stabilized with BSA before lyophilization.				

Introduction

The green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is a versatile marker for monitoring physiological processes, visualizing protein localization, and detecting transgenic expression.^{1–5} Invitrogen offers the anti-GFP antibody as a rabbit polyclonal, monoclonal, or IgG fraction, two mouse monoclonal antibodies, and a chicken IgY fraction. All six anti-GFP antibodies are suited for detection of native GFP, GFP variants, and most GFP fusion proteins by western blot analysis while the rabbit and mouse antibodies are also useful for immunoprecipitation. The anti-GFP rabbit polyclonal antibody is raised against GFP isolated directly from *Aequorea victoria* (Table 2). The rabbit anti-GFP antibody is available as a complete antiserum (A6455) or as an IgG fraction purified by ion-exchange chromatography (A11122). Like our multi-purpose polyclonal anti-GFP antibody, the rabbit anti-GFP monoclonal antibody (G10362) is raised against full-length GFP; it is suitable for immunoprecipitation, immunohistochemistry, and western blotting. Anti-GFP mouse monoclonal antibody 3E6 (A11120) is useful for immunoprecipitation, immunocytochemical

localization, and immunosorbent assays (ELISA). Anti-GFP mouse monoclonal antibody 11E5 (A11121) is optimized for western analysis, allowing colorimetric detection of as little as 10 ng of GFP or GFP-fusion proteins, or chemiluminescent detection of picogram quantities. The chicken anti-GFP antibody (A10262) is raised against GFP isolated directly from *Aequorea victoria* (Table 2), and the IgY fraction is purified by affinity purification. The chicken IgY lacks a classic “Fc” domain and does not bind to mammalian IgG Fc receptors, resulting in lower backgrounds during immunostaining protocols. The chicken IgY is also antigenically different from the mammalian IgG, allowing you to perform double immunostaining experiments using antibodies from multiple species.

At the time of preparation, the products are certified to be free of unconjugated dyes. They are tested in a cytological experiment to ensure low nonspecific staining.

Table 2. Anti-GFP antibodies.

Catalog no.	Host	Amount	Application †	Type
A6455	Rabbit	100 µL	IP, IHC, WB	Serum
A10262	Chicken	100 µL*	ICC, WB	IgY fraction
A11122	Rabbit	100 µL*	IP, IHC, WB	IgG fraction
A11120	Mouse	100 µg	IP, IHC	mAb, IgG _{2a}
A11121	Mouse	100 µg	WB	mAb, IgG ₁
G10362	Rabbit	100 µg	ELISA, IP, IHC, WB, Flow	mAb

* 2 mg/mL. †Immunoprecipitation (IP), immunohistochemistry (IHC), western blot (WB), immunocytochemistry (ICC), and flow cytometry (Flow).

Before You Begin

Preparing the Anti-GFP Monoclonal Antibody Stock Solutions

To prepare 0.2 mg/mL stock solutions, reconstitute the lyophilized antibodies in 0.5 mL of phosphate-buffered saline (PBS), pH 7.4. You may store these solutions for up to 3 months at 4°C with the addition of 2 mM sodium azide.

Dilution and Centrifugation

Because protocols vary with application, empirically determine the appropriate dilution of anti-GFP. For initial experiments, we recommend trying dilutions ranging from 1:200 to 1:2,000 for immunocytochemical (ICC) applications and western blot analysis with the exception of the Abfinity™ antibody, which has a 1:20 to 1:200 dilution range for ICC and western blot analysis. It is a good practice to centrifuge the protein conjugate solutions briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step eliminates any protein aggregates that may have formed during storage, and reduces nonspecific background staining.

Experimental Protocol for Immunoprecipitation

Use the following immunoprecipitation protocol with rabbit and mouse anti-GFP antibodies but not with chicken anti-GFP antibody. Read the entire protocol before starting.

Materials Required but Not Provided

- 1X Phosphate-buffered saline, pH 7.4 (PBS, Cat. no. 10010-031)
- Blocking buffer: 5% Normal Goat Serum (NGS) in PBS, pH 7.4
- Wash buffer: 5% NGS in PBS, 1% Triton® X-100, 3% Bovine Serum Albumin (BSA)
- Sheep anti-rabbit magnetic beads (Cat. no. 112-03)
- Sheep anti-mouse magnetic beads (Cat. no. 110-31)
- Magnetic rack
- NuPAGE® LDS Sample Buffer (Cat. no. NP0007) or an equivalent SDS sample buffer
- NuPAGE® gel or equivalent SDS gel

Preparing Magnetic Beads

For each sample, you will need 50 µL of sheep anti-rabbit or sheep anti-mouse magnetic beads.

- 1.1 Transfer the required amount of beads to a clean microcentrifuge tube.
- 1.2 Place the tubes containing beads on a magnetic rack for 1 minute. Carefully discard the supernatant.
- 1.3 Resuspend the beads in 500 µL of PBS by pipetting gently up and down. Remove the tubes from the magnetic rack and rotate the tubes gently for 10 minutes.
- 1.4 Place the tubes on a magnetic rack for 1 minute and discard the supernatant.
- 1.5 Repeat the wash steps 1.3–1.4 two more times. Resuspend the beads in 500 µL of PBS. **Do not allow the beads to dry out.**

Immunoprecipitation Protocol

- 2.1 Preclear the lysate by combining 50 µL of sheep anti-rabbit or sheep anti-mouse magnetic beads with 30 µg of sample lysate in a microcentrifuge tube. Incubate the tube at 4°C with gentle rotation for 1 hour.
- 2.2 Place tubes on a magnetic rack for 1 minute and transfer the supernatant into a new microcentrifuge tube placed on ice.
- 2.3 To the precleared lysate, add 5% NGS in PBS for blocking, and then add primary anti-GFP antibody diluted in PBS to a final concentration of 0.2 µg/mL. Incubate the sample at 4°C overnight with gentle rotation.
- 2.4 Place the tubes containing the sheep anti-rabbit (for rabbit antibody) or sheep anti-mouse (for mouse antibody) magnetic beads (prepared in steps 1.1–1.5) on a magnetic rack for 1 minute and discard the supernatant.

Add the sheep anti-rabbit or sheep anti-mouse magnetic beads to the samples. Incubate the samples at room temperature for 1 hour with gentle rotation.

- 2.5 Place the tubes on a magnetic rack for 1 minute. Discard the supernatant.

- 2.6 Remove the tubes from the magnetic rack, and resuspend the beads in Wash buffer (5% NGS in PBS, 1% Triton® X-100, 3% BSA) by pipetting gently up and down. Rotate the tubes gently for 10 minutes.
- 2.7 Place the tubes on a magnetic rack for 1 minute. Discard the supernatant.
- 2.8 Repeat the wash steps 2.6–2.7 two more times.
- 2.9 Add 25 µL of 1X SDS sample buffer to the beads. Heat the samples at 100°C for 5 minutes. If you are using NuPAGE® LDS Sample Buffer, heat the samples at 70°C for 10 minutes.
- 2.10 Place the tubes on a magnetic rack for 1 minute. Transfer the supernatant to a clean, microcentrifuge tube, and analyze the supernatant using SDS-PAGE.

Experimental Protocol for Western Detection

Use the following western detection protocol with rabbit, mouse, and chicken anti-GFP antibodies. Be sure to use enough solution in an appropriate container to completely cover the transfer membrane with the solution. Do not allow the membrane to fold or bend. Do not allow any part of the membrane to dry out during the western protocol.

Materials Required but Not Provided

- 1X Phosphate-buffered saline (PBS, Cat. no. 10010-031)
- Tris buffered saline with 0.05% Tween-20 (TBST)
- Conjugated anti-mouse IgG antibody, conjugated anti-rabbit IgG antibody, or conjugated anti-chicken IgG antibody (depending on the primary antibody species used)
- Blocking buffer: 5% (w/v) nonfat dry milk in TBST
- Transfer membrane (nitrocellulose or PVDF)
- Orbital shaker platform
- Trays

Western Detection Protocol

- 3.1 After transferring the proteins to the nitrocellulose or PVDF membrane, rinse the membrane once with PBS.

Note: If the PVDF membrane is dry, place the PVDF membrane in 100% methanol for 30 seconds and then place the membrane in TBST for 1 minute. Decant the TBST.
- 3.2 Place the membrane in the appropriate volume of Blocking buffer in a plastic dish. Incubate the membrane for 1 hour at room temperature on a shaker with gentle agitation. Decant the Blocking buffer.
- 3.3 Wash the membrane twice with TBST with gentle agitation for 1 minute each.
- 3.4 Prepare a dilution of the anti-GFP antibody in TBST as described below:
 - Dilute the rabbit anti-GFP antibody 1:1,000
 - Dilute the ABfinity™ antibody 1:100
 - Dilute the mouse anti-GFP antibody 1:1,000
 - Dilute the chicken anti-GFP antibody 1:1,000 in TBST to obtain a final antibody concentration of 2.0 µg/mL.

- 3.5 Decant the TBST and add the diluted anti-GFP antibody solution from step 3.4 to the membrane. Incubate the membrane for 1 hour at room temperature on a shaker with gentle agitation. Decant the antibody solution.
- 3.6 Wash the membrane twice with TBST with gentle agitation for 1 minute each.
- 3.7 Prepare the appropriate conjugated secondary antibody in TBST according to the manufacturer's recommendations.
- 3.8 Decant the TBST. Add the diluted secondary antibody solution to the membrane and incubate for 1 hour at room temperature on a shaker with gentle agitation. Decant the antibody solution.
- 3.9 Wash the membrane three times with TBST with gentle agitation for 5–10 minutes each.
- 3.10 Continue processing the blot using the appropriate method for the type of conjugate used (e.g., horseradish peroxidase or alkaline phosphatase) until the blot is ready for imaging and detection.

Experimental Protocol for Immunocytochemistry

The following protocol is designed for immunocytochemistry using the anti-GFP, chicken IgY fraction (A10262). Read the entire protocol before starting.

Materials Required but Not Provided

- 1X Dulbecco's Phosphate-buffered saline (D-PBS, Cat. no. 14190-136)
- Fixative solution: 4% Formaldehyde solution in PBS, pH 7.4
- Permeabilizing solution: 0.25% Triton® X-100 in PBS, pH 7.4
- Blocking solution: 5% Normal Goat serum in PBS, pH 7.4
- Conjugated secondary anti-chicken IgG antibody for detection
- 1X Phosphate-buffered saline (PBS) pH 7.4 (Cat. no. 10010-031)

Preparing Cells

Culture mammalian cells on cover slips in the appropriate medium to ~75% confluency.

Immunocytochemistry Protocol

- 4.1 Remove the media from the cells grown on cover slips. Rinse the cells twice for 1 minutes each in D-PBS.
- 4.2 Fix the cells in Fixative solution (4% formaldehyde in PBS) for 30 minutes at room temperature with gentle agitation in the dark. Remove the solution.
- 4.3 Wash the cells twice in PBS for 1 minute each with gentle agitation. Remove the PBS.
- 4.4 Permeabilize the specimen with Permeabilization solution (0.25% Triton® X-100 in PBS) for 5 minutes at room temperature with gentle agitation in the dark. Remove the solution.
- 4.5 Wash the cells twice in PBS for 1 minute each with gentle agitation. Remove the PBS.

- 4.6 Add Blocking solution (5% Normal Goat Serum in PBS, pH 7.4) to the cells. Incubate for 1 hour at room temperature with gentle agitation. Remove the solution.
- 4.7 Wash the cells twice in PBS for 1 minute each with gentle agitation.
- 4.8 Prepare a 1:400 dilution of anti-GFP chicken antibody in PBS to obtain a final antibody concentration of 5.0 µg/mL.
- 4.9 Remove the PBS and add the diluted primary antibody solution to the cells. Incubate for 1 hour at room temperature with gentle agitation. Remove the solution.
- 4.10 Wash the cells twice in PBS for 1 minute each with gentle agitation.
- 4.11 Prepare the appropriate conjugated secondary antibody in PBS according to the manufacturer's recommendations.
- 4.12 Remove the PBS and add the diluted secondary antibody solution to the cells. Incubate for 1 hour at room temperature with gentle agitation. Remove the solution.
- 4.13 Wash the cells twice in PBS for 2 minutes each with gentle agitation. After the final wash, add PBS to the sample.

The sample is now ready for imaging and detection using an appropriate method of choice.

References

1. *Methods in Enzymology*, Vol. 302, P.M. Conn, Ed., Academic Press (1999);
2. *Annu Rev Biochem* 67, 509 (1998);
3. *Nat Biotechnol* 15, 961 (1997);
4. *Nature* 369, 400 (1994);
5. *Science* 263, 802 (1994).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
A6455	anti-green fluorescent protein, rabbit serum (anti-GFP, serum)	100 µL
A10262	anti-green fluorescent protein, chicken IgY fraction	100 µL
A11120	anti-green fluorescent protein, mouse monoclonal 3E6 (anti-GFP, mAb 3E6)	100 µg
A11121	anti-green fluorescent protein, mouse monoclonal 11E5 (anti-GFP, mAb 11E5)	100 µg
A11122	anti-green fluorescent protein, rabbit IgG fraction (anti-GFP, IgG) *2 mg/mL*	100 µL
G10362	GFP, ABfinity™ recombinant rabbit monoclonal antibody - unconjugated (anti-GFP, rabbit mAb)	100 µg
Related Products		
110-31	Dynabeads® Sheep anti-mouse beads	5 mL
112-03D	Dynabeads® Sheep anti rabbit beads	2 mL
14190-136	Dulbecco's Phosphate Buffered Saline (D-PBS) (1X), liquid, without calcium, magnesium, or phenol red.	1000 mL
10010-031	Phosphate-Buffered Saline (PBS) 7.4 (1X), liquid	1000 mL
R10367	RFP, rabbit polyclonal antibody - unconjugated (anti-RFP rabbit IgG)	100 µg

Visit www.invitrogen.com/antibody for details on antibody research reagents and tools.

A variety of products is available for western blotting including precast NuPAGE® gels, premade buffers, protein standards, blotting membranes, and western detection kits. Visit www.invitrogen.com/1D for details.

Contact Information

Molecular Probes, Inc.

29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:

6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:

Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:

8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probetech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology

The manufacture, use, sale or import of this product may be subject to one or more patents or pending applications owned or licensed by Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity) in a manner consistent with the accompanying product literature. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the most restrictive terms apply. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents that are owned or controlled by Life Technologies Corporation and/or Molecular Probes, Inc. which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2010, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.