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by life technologies™

Anti-GFP Antibodies

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
Anti-GFP, rabbit polyclonal serum (Cat. no. A6455)	100 µL, with 0.01% thimerosal	Not applicable	• 2–8°C • Protect from light	When stored undiluted as directed, products are stable for at least 3 months. 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, rabbit IgG fraction (Cat. no. A11122)	100 µL	2 mg/mL solution in PBS, pH 7.2, 5 mM azide		
Anti-GFP, chicken IgY fraction (Cat. no. A10262)				
Anti-GFP mouse monoclonals 3E6 (Cat. no. A11120, isotype IgG_{2a}) and 11E5 (Cat. no. 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) (Cat. no. 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] The anti-GFP antibody is available as a rabbit polyclonal, monoclonal, or IgG fraction, two mouse monoclonal antibodies, and a chicken IgY fraction (see page 7 for ordering information). 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 (Cat. no. A6455) or as an IgG fraction purified by ion-exchange chromatography (Cat. no. A11122). Like our multi-purpose polyclonal anti-GFP antibody, the rabbit anti-GFP monoclonal antibody (Cat. no. G10362) is raised against full-length GFP; it is suitable for immunoprecipitation, immunohistochemistry, and

western blotting. Anti-GFP mouse monoclonal antibody 3E6 (Cat. no. A11120) is useful for immunoprecipitation, immunocytochemical localization, and immunosorbent assays (ELISA). Anti-GFP mouse monoclonal antibody 11E5 (Cat. no. 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 (Cat. no. 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

* Immunoprecipitation (IP), immunohistochemistry (IHC), western blot (WB), immunocytochemistry (ICC), and flow cytometry (Flow).
**2 mg/mL.
† G10362 has not been demonstrated to work with formalin-fixed, paraffin-embedded (FFPE) tissue samples.

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 1:2000 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)
- Dynabeads® M-280 Sheep Anti-Rabbit IgG (Cat. no. 11203D)
- Dynabeads® Sheep-Anti Mouse IgG (Cat. no. 11031)
- 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.

2.5 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.

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

2.7 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.

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

2.9 Repeat the wash steps 2.7–2.8 two more times.

2.10 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.

2.11 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 solution (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 1:1000
- Dilute the ABfinity™ antibody 1:100
- Dilute the mouse anti-GFP antibody 1:1000
- Dilute the chicken anti-GFP antibody 1:1000 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 (Cat. no. 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.

4.1 Remove the media from the cells grown on cover slips. Rinse the cells twice for 1 minute 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 solution 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		
11031	Dynabeads® Sheep anti-mouse beads	5 mL
11203D	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

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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.lifetechnologies.com for details.

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