

Anti-GFP Antibodies

Quick Facts

Storage upon receipt:

- 4°C or -20°C in aliquots (A-6455, A-11122, A-21311, A-21312)
- -20°C (A-11120, A-11121)
- Desiccate (A-11120, A-11121)
- Protect from light (A-21311, A-21312)

Abs/Em: See Table 2

Table 1. Anti-GFP antibodies.

Catalog#	Host	Amount	Application †	Type
A-6455	Rabbit	100 µL	IP, IHC, WB	Serum
A-11122	Rabbit	100 µL *	IP, IHC, WB	IgG fraction
A-11120	Mouse	100 µg	IP, IHC	mAb, IgG _{2a}
A-11121	Mouse	100 µg	WB	mAb, IgG ₁

* 2 mg/mL. † Immunoprecipitation (IP), immunohistochemistry (IHC) and Western blot (WB).

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 Molecular Probes offers both a rabbit polyclonal antibody and two mouse monoclonal antibodies for detection of native GFP, GFP variants and most GFP fusion proteins by Western blot analysis or for immunoprecipitations. Molecular Probes' anti-GFP rabbit polyclonal antibody is raised against GFP isolated directly from *Aequorea victoria*, see Table 1. The rabbit anti-GFP antibody is available as a complete antiserum (A-6455) or as an IgG fraction purified by ion-exchange chromatography (A-11122). Anti-GFP mouse monoclonal antibody 3E6 (A-11120) is useful for immunoprecipitation, immunocytochemical localization and immunosorbent assays (ELISA). Anti-GFP mouse monoclonal antibody 11E5 (A-11121) 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.

Molecular Probes offers two Alexa Fluor® dye-conjugates made from the rabbit anti-GFP IgG fraction. The Alexa Fluor dyes provide for extraordinarily bright antibody conjugates. The approximate absorption and fluorescence emission maxima for each conjugate are shown in Table 2.

Materials

Anti-GFP Polyclonal Antibodies

Anti-GFP rabbit serum is supplied in a unit size of 100 µL containing 0.01% thimerosal as a preservative. Anti-GFP, IgG fraction is supplied in a unit size of 100 µL as a 2 mg/mL solution in phosphate-buffered saline (PBS), pH 7.2, containing 0.01% thimerosal as a preservative.

The fluorophore-labeled rabbit anti-GFP IgG antibodies are supplied in unit sizes of 100 µL as 2 mg/mL solutions in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide. The degree of labeling for each conjugate is typically 2–8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the product label. At the time of preparation, the products are certified to be free of unconjugated dyes and are tested in a cytological experiment to ensure low nonspecific staining.

When these products are stored undiluted at 4°C, they are stable for at least three months. For longer storage, divide the solution into single-use aliquots and freeze at -20°C. Frozen aliquots are stable for at least six months. Protect the fluorescent conjugates from light. AVOID REPEATED FREEZING AND THAWING.

Anti-GFP Monoclonal Antibodies

Anti-GFP mouse monoclonals 3E6 (A-11120, isotype IgG_{2a}) and 11E5 (A-11121, isotype IgG₁) are purified from mouse hy-

Table 2. Alexa Fluor dye-labeled rabbit anti-GFP conjugates.

Catalog#	Host	Fluorophore	Abs/Em *	Application †	Type
A-21311	Rabbit	Alexa Fluor 488	495/519	IHC, WB	IgG fraction
A-21312	Rabbit	Alexa Fluor 594	590/617	IHC, WB	IgG fraction

* Approximate absorption (Abs) and fluorescence emission (Em) maxima. † Immunohistochemistry (IHC) and Western blot (WB).

bridoma supernatants by protein G chromatography followed by extensive dialysis. Upon receipt, the 100 µg units of lyophilized antibody should be stored desiccated at -20°C. To prepare stock solutions (0.2–0.5 mg/mL), reconstitute 100 µg units of lyophilized antibodies in 0.2–0.5 mL of phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin (BSA). These solutions may be stored for up to 2 weeks at 4°C with the addition of 2 mM sodium azide.

Application

Because protocols vary with application, the appropriate dilution of anti-GFP should be determined empirically. For initial experiments, we recommend trying dilutions ranging from 1:200 to 1:2000 for immunohistochemical applications and for Western-blot analysis.

It is a good practice to centrifuge the protein conjugate solutions briefly in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For fluorophore-labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunohistochemical applications.¹

Immunoprecipitation Using mAb 3E6 (A-11120)

Materials Required

- Protein A- or protein G-conjugated agarose (note A)
- Centrifuge
- Wash Buffer A1: 50 mM Tris, 0.15 M NaCl, 1 mM EDTA, 0.1% NP-40 (nonylphenoxy polyethoxyethanol), pH 7.5
- Wash Buffer A2: 50 mM Tris, 0.15 M NaCl, 0.1% NP-40, pH 7.5
- Wash Buffer A3: 50 mM Tris, 0.1% NP-40, pH 7.5
- Apparatus and reagents for SDS-PAGE

Protocol

1.1 Add 25 µL of homogeneous protein A-agarose (note A) to 500 µL of GFP-containing sample. Incubate for at least 3 hours at 4°C with gentle agitation.

1.2 Centifuge for 20 seconds at 12,000 × g. Transfer the supernatant to a clean microfuge tube.

1.3 Add 1–5 µg of mAb 3E6 to the sample (note B). Incubate for 1 hour at 4°C with gentle agitation.

1.4 Add 25 µL of homogeneous protein A-agarose to the sample (note B).

1.5 Incubate for at least 3 hours (or overnight) at 4°C with gentle agitation. Centifuge for 20 seconds at 12,000 × g. Carefully remove the supernatant and resuspend the precipitate in 1 mL of Wash Buffer A1.

1.6 Incubate for 20 minutes at 4°C with gentle agitation. Centifuge for 20 seconds at 12,000 × g. Carefully remove the supernatant and wash again with 1 mL of Wash Buffer A1.

1.7 Incubate for 20 minutes at 4°C with gentle agitation. Centifuge for 20 seconds at 12,000 × g. Carefully remove the supernatant and wash with 1 mL of Wash Buffer A2.

1.8 Incubate for 20 minutes at 4°C with gentle agitation. Centifuge for 20 seconds at 12,000 × g. Carefully remove the supernatant and add 1 mL of Wash Buffer A3.

1.9 Incubate for 20 minutes at 4°C with gentle agitation. Centifuge for 20 seconds at 12,000 × g. Completely remove the supernatant.

1.10 Add 25–75 µL of SDS sample buffer to the precipitate, heat to 100°C for 3 minutes then centrifuge for 20 seconds at 12,000 × g.

1.11 Transfer the supernatant to a clean microfuge tube and analyze by SDS-PAGE according to standard protocols.²

Western Detection Using mAb 11E5 (A-11121)

Materials Required

- Phosphate-buffered saline (PBS)
- Alkaline phosphatase-conjugated anti-mouse IgG antibody
- NBT/BCIP detection reagent: 0.46 mM NBT, 0.43 mM BCIP in 0.1 M Tris, 0.1 M NaCl, 0.05 M MgCl₂, pH 9.5. NBT and BCIP are supplied in Molecular Probes' NBT/BCIP Reagent Kit (N-6547)
- Blocking Solution: 5% (w/v) nonfat dry milk, 0.05% Tween[®] 20, 0.02% sodium azide in PBS
- Wash Buffer B1: PBS, 0.1% Tween 20
- Wash Buffer B2: 50 mM Tris, 0.15 M NaCl, 0.1% Tween 20, pH 7.5
- Wash Buffer B3: 50 mM Tris, 0.15 M NaCl, pH 7.5
- 20 mM EDTA solution
- Blot transfer membrane

Protocol

2.1 Rinse the membrane several times with PBS. Add Blocking Solution and incubate for at least 1 hour at room temperature with agitation.

2.2 Remove the blot from the Blocking Solution and wash twice for 5 minutes each in PBS. Add 10 mL of a 0.5 µg/mL working solution of mAb 11E5 that has been prepared in Blocking Solution.

2.3 Incubate for at least 1 hour at room temperature with agitation. After incubation, wash the blot four times for 10 minutes each in Wash Buffer B1.

2.4 Add the alkaline phosphatase-conjugated secondary antibody, diluted in blocking buffer according to the manufacturer's recommendations.

2.5 Incubate for at least 1 hour at room temperature with agitation. After incubation, wash the blot four times for 10 minutes each in Wash Buffer B2.

2.6 Rinse the blot twice for 2–5 minutes each in 100–150 mL of Wash Buffer B3. Thorough washing is essential to remove all phosphate prior to the addition of the NBT/BCIP detection reagent.

2.7 Immerse the blot in the NBT/BCIP detection reagent with periodic gentle agitation.

2.8 Continue development until visibly dark bands appear (this may take several minutes). Stop the reaction by immersing the blot in 20 mM EDTA solution.

Notes

[A] Because monoclonal 3E6 is a mouse IgG_{2a}, either protein A– or protein G–conjugated agarose can be used. Protein A–agarose is specified in the protocol description only for example.

[B] The optimal ratios of GFP-containing sample, anti-GFP mAb 3E6 and protein A–agarose may vary from sample to sample, depending on the sample concentration and the amount of protein A (or protein G) coupled to the agarose. Concentrations and amounts specified in this protocol are provided as an example. Optimal ratios can be determined by performing a preliminary small scale precipitation.

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); 6. *Short Protocols in Molecular Biology*, 2nd Edition, F.M. Ausubel, Ed., John Wiley & Sons (1992) pp 10.7–10.15.

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
A-11120	anti-green fluorescent protein, mouse monoclonal 3E6 (anti-GFP, mAb 3E6)	100 µg
A-11121	anti-green fluorescent protein, mouse monoclonal 11E5 (anti-GFP, mAb 11E5)	100 µg
A-21311	anti-green fluorescent protein, rabbit IgG fraction, Alexa Fluor® 488 conjugate (anti-GFP, IgG, Alexa Fluor® 488 conjugate) *2 mg/mL*	100 µL
A-21312	anti-green fluorescent protein, rabbit IgG fraction, Alexa Fluor® 594 conjugate (anti-GFP, IgG, Alexa Fluor® 594 conjugate) *2 mg/mL*	100 µL
A-11122	anti-green fluorescent protein, rabbit IgG fraction (anti-GFP, IgG) *2 mg/mL*	100 µL
A-6455	anti-green fluorescent protein, rabbit serum (anti-GFP, serum)	100 µL

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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 Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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