Anti-GFP Antibody Conjugates

Table 1. Contents and storage information.

Material*	Amount	Concentration	Storage	Stability
Anti-GFP, rabbit IgG fraction, biotin-XX conjugate (A10259)	100 µL	2 mg/mL in 0.1 M sodium phosphate buffer, pH 7.5, 0.1 M NaCl, 5 mM sodium azide	• 2–6°C • Avoid freeze-thaw cycles	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, chicken IgY fraction, biotin-XX conjugate (A10263)	100 µL	2 mg/mL in 0.1 M sodium phosphate buffer, pH 7.5, 0.1 M NaCl, 5 mM sodium azide		
Anti-GFP, rabbit IgG fraction, fluorophore-labeled (A21311, A21312, A31851, A31852)	100 µL	2 mg/mL solutions in 0.1 M sodium phosphate, buffer pH 7.5, 0.1 M NaCl, 5 mM sodium azide	• 2–6°C • Protect from light • Avoid freeze-thaw cycles	
Anti-GFP, rabbit IgG fraction, horseradish peroxidase (HRP) conjugate (A10260)	200 µg	Not applicable	• ≤–20°C • Dessicate	When stored dry as directed, products are stable for at least 6 months.

*The exact degree of labeling for each conjugate is indicated on the product label.

Approximate fluorescence excitation and emission, in nm: See Table 3.

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 conjugates as rabbit IgG fraction and chicken IgY fraction conjugated to biotin-XX, horseradish peroxidase (HRP), or fluorophore labeled. All anti-GFP antibody conjugates are suited for detection of native GFP, GFP variants, and most GFP fusion proteins by western blot analysis and immunocytochemistry. The anti-GFP rabbit polyclonal antibody is raised against GFP isolated directly from *Aequorea victoria* and the IgG fraction is purified by ion-exchange chromatography. The purified IgG is then conjugated to biotin-XX (A10259) or HRP (A10260) (Table 2). The chicken anti-GFP IgY fraction is purified by affinity purification and the purified IgY is then conjugated to biotin-XX (A10263). 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.

The anti-GFP biotin-XX conjugates contain 3–7 moles of biotin per mole of antibody.

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.

Several Alexa Fluor® dye–conjugates made from the rabbit anti-GFP IgG fraction are also available from Invitrogen. The Alexa Fluor® dyes provide for extraordinarily bright antibody conjugates. The approximate fluorescence excitation and emission maxima for each conjugate are shown in Table 3.

Table 2. Anti-GFP antibody conjugates.

Catalog no.	Host	Amount	Application†	Type
A10259	Rabbit	100 µL	ICC, WB	IgG fraction
A10260	Rabbit	200 µg	ICC, WB	IgG fraction
A10263	Chicken	100 µL	ICC, WB	IgY fraction

†Immunoprecipitation (IP), immunohistochemistry (IHC), western blot (WB), and immunocytochemistry (ICC).

Table 3. Alexa Fluor® dye–labeled rabbit anti-GFP conjugates.

Catalog no.	Fluorophore	Ex/Em*
A21311	Alexa Fluor 488	495/519
A31851	Alexa Fluor 555	555/565
A21312	Alexa Fluor 594	590/617
A31852	Alexa Fluor 647	650/668

*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm.

Before You Begin

Preparing the Anti-GFP Rabbit IgG-HRP Conjugate stock solution

To prepare 0.4 mg/mL stock solutions, reconstitute the lyophilized antibody in 0.5 mL of phosphate-buffered saline (PBS), pH 7.4. These solutions may be stored 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 that range from 1:200 to 1:2000 for immunocytochemical applications and western blot analysis. For fluorophore-labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunocytochemical applications.[1]

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, thereby reducing nonspecific background staining.

Qdot® Streptavidin Conjugates

Detailed protocols for using the anti-GFP antibody biotin-XX conjugates with Qdot® Streptavidin Conjugates are included in the Qdot® Streptavidin Conjugates handbook (MP 19000) available for downloading from www.lifetechnologies.com.

Experimental Protocol for Western Detection

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

Please read the entire protocol before starting.

Materials required but not provided

- 1X Phosphate-buffered saline (PBS, Invitrogen Cat. no. 10010-031)
- Tris buffered saline with 0.05% Tween-20 (TBST)
- Streptavidin conjugate of choice (for example, fluorescent, HRP, alkaline-phosphatase) for use with biotin-XX conjugate (A10259 or A10263)
- Chromogenic or chemiluminescent substrate/reagents for use with HRP conjugate (A10260)
- Blocking buffer: 5% (w/v) nonfat dry milk in TBST
- Transfer membrane (nitrocellulose or PVDF)
- Orbital shaker platform
- Trays

Western detection protocol

1.1 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, then place the membrane in TBST for 1 minute. Decant TBST.

1.2 Place membrane in the appropriate volume of Blocking buffer in a plastic dish. Incubate for 1 hour at room temperature with gentle agitation. Decant Blocking buffer.

1.3 Wash the membrane twice with TBST with gentle agitation for 1 minute each.

1.4a If using anti-GFP biotin-XX conjugate (A10259 or A10263), prepare a dilution of the anti-GFP antibody-biotin-XX conjugate as described below:

- Dilute the rabbit (A10259) or chicken (A10263) anti-GFP antibody-biotin-XX conjugate 1:500 in TBST to obtain a final antibody concentration of 4 µg/mL.
- To the **same tube** containing the diluted anti-GFP antibody-biotin conjugate, add the appropriate volume of streptavidin conjugate to obtain the manufacturer's recommended final streptavidin conjugate concentration. Mix gently.

1.4b If using the rabbit anti-GFP antibody-HRP conjugate (A10260), dilute the conjugate 1:2000 in TBST to obtain a final antibody concentration of 2 µg/mL.

1.4c If using the rabbit anti-GFP antibody-Alexa Fluor® conjugates (A21311, A31851, A21312, or A31852), dilute the conjugate 1:2000 in PBS to obtain a final antibody concentration of 1 µg/mL.

1.5 Decant TBST.

For anti-GFP antibody-biotin-XX conjugates (A10259 or A10263), add the diluted antibody solution from step **1.4a.**

For anti-GFP antibody-HRP conjugate (A10260), add the diluted antibody solution from step **1.4b**.

For anti-GFP antibody-Alexa Fluor® conjugates, add the diluted antibody solution from step **1.4c**.

Incubate for 1 hour at room temperature with gentle agitation. Decant antibody solution.

1.6 Wash the membrane twice with TBST with gentle agitation for 1 minute each. Decant TBST.

1.7 Wash the membrane three times with TBST with gentle agitation for 5–10 minutes each.

1.8 If using fluorescent conjugates, the blot is ready for imaging and detection using an appropriate method of choice. See Table 3 for approximate fluorescence excitation and emission in nm for Alexa Fluor® conjugates.

If using biotin-XX or HRP conjugate, continue processing the blot according to the appropriate procedure to prepare the blot for imaging and detection.

Note: For western detection, we have tested Alexa Fluor® 647 conjugates (goat anti-mouse, goat anti-rabbit, and goat anti-chicken) on Fuji FLA 3000 and Kodak IS2000 MI imaging platforms. Depending on the imaging platform and the dye used, you may need to optimize the settings to obtain the best results.

Experimental Protocol for Immunocytochemistry

The following protocol is designed for immunocytochemistry using rabbit and chicken anti-GFP antibody conjugates. This immunocytochemistry protocol was developed using HeLa and U2-OS cells. This protocol has not been tested with paraffin-embedded sections.

Please read the entire protocol before starting.

Materials required but not provided

- 1X Dulbecco's Phosphate-buffered saline (D-PBS, Invitrogen 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
- Streptavidin conjugate of choice (for example, fluorescent, HRP, alkaline-phosphatase) for use with biotin-XX conjugate (A10259 or A10263)
- TSA™ kits for use with HRP conjugate (A10260)
- 1X Phosphate-buffered saline (PBS) pH 7.4 (Invitrogen Cat. no. 10010-031)

Preparing cells

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

Immunocytochemistry protocol

2.1 Remove media from cells grown on cover slips. Rinse cells twice for 1 minute each in D-PBS.

2.2 Fix cells in Fixative solution (4% formaldehyde in PBS) for 30 minutes at room temperature with gentle agitation in the dark. Remove the solution.

2.3 Wash cells twice in PBS for 1 minute each with gentle agitation. Remove PBS.

2.4 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.

2.5 Wash cells twice in PBS for 1 minute each with gentle agitation. Remove PBS.

2.6 Add Blocking solution (5% Normal Goat Serum in PBS pH 7.4). Incubate for 1 hour at room temperature with gentle agitation. Remove the solution.

2.7 Wash cells twice in PBS for 1 minute each with gentle agitation.

2.8a If using anti-GFP biotin-XX conjugates (A10259 or A10263), prepare a dilution of the conjugate as described below:

- Dilute the rabbit (A10259) or chicken (A10263) anti-GFP antibody-biotin-XX conjugate 1:400 in PBS to obtain a final antibody concentration of 5 µg/mL.
- To the **same tube** containing the diluted anti-GFP antibody-biotin conjugate, add the appropriate volume of streptavidin conjugate to obtain the manufacturer's recommended final streptavidin concentration. Mix gently.

2.8b If using the rabbit anti-GFP antibody-HRP conjugate (A10260), dilute the conjugate 1:400 in PBS to obtain a final antibody concentration of 5 µg/mL.

2.8c If using rabbit anti-GFP antibody-Alexa Fluor® conjugates (A21311, A31851, A21312, A31852), dilute the conjugate 1:400 in PBS to obtain a final antibody concentration of 5 µg/mL.

2.9 Remove PBS.

For anti-GFP antibody-biotin-XX conjugates (A10259 or A10263), add the diluted antibody solution from step **2.8a.**

For anti-GFP antibody-HRP conjugate (A10260), add the diluted antibody solution from step **2.8b**.

For anti-GFP antibody-Alexa Fluor® conjugates, add the diluted antibody solution from step **2.8c**.

Incubate for 1 hour at room temperature with gentle agitation. Decant antibody solution.

2.10 Wash cells twice in PBS for 2 minutes each with gentle agitation. After the final wash, add PBS to the sample.

Continue processing HRP conjugate sample with the appropriate detection protocol. The sample is now ready for imaging and detection.

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
A10259	Anti-green fluorescent protein, rabbit IgG fraction, biotin-XX conjugate	100 µL
A10260	Anti-green fluorescent protein, rabbit IgG fraction, horseradish peroxidase conjugate	100 µL
A10263	Anti-green fluorescent protein, chicken IgY fraction, biotin-XX conjugate	100 µL
A21311	Anti-green fluorescent protein, rabbit IgG fraction, Alexa Fluor® 488 conjugate (anti-GFP, IgG, Alexa Fluor® 488 conjugate) *2 mg/mL*	100 µL
A31851	Anti-green fluorescent protein, rabbit IgG fraction, Alexa Fluor® 555 conjugate (anti-GFP, IgG, Alexa Fluor® 555 conjugate *2 mg/mL*	100 µL
A21312	Anti-green fluorescent protein, rabbit IgG fraction, Alexa Fluor® 594 conjugate (anti-GFP, IgG, Alexa Fluor® 594 conjugate) *2 mg/mL*	100 µL
A31852	Anti-green fluorescent protein, rabbit IgG fraction, Alexa Fluor® 647 conjugate (anti-GFP, IgG, Alexa Fluor® 647 conjugate) *2 mg/mL*	100 µL
Related Products		
S32354	Streptavidin, Alexa Fluor® 488 conjugate *2 mg/mL*	0.5 mL
S32355	Streptavidin, Alexa Fluor® 555 conjugate *2 mg/mL*	0.5 mL
S32356	Streptavidin, Alexa Fluor® 594 conjugate *2 mg/mL*	0.5 mL
S32357	Streptavidin, Alexa Fluor® 647 conjugate *2 mg/mL*	0.5 mL
S911	Streptavidin, horseradish peroxidase conjugate	1 mg
T20912	TSA™ Kit #2 *with HRP–goat anti-mouse IgG and Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
T20916	TSA™ Kit #6 *with HRP–goat anti-mouse IgG and Alexa Fluor® 647 tyramide* *50–150 slides*	1 kit
T20922	TSA™ Kit #12 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
T20932	TSA™ Kit #22 *with HRP–streptavidin and Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
Q10121MP	Qdot® 655 streptavidin conjugate *1 µM solution*	200 µL
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

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.

Contact Information

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European Headquarters
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese Headquarters
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
Fax: +81 3 5730 6519
Email: jpinfo@invitrogen.com

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