

Goat Anti–Rabbit IgG Antibodies

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage Upon Receipt	Stability
Whole antibodies	0.5 mL	2 mg/mL in 0.1 M sodium phosphate, 0.1 M NaCl, 5 mM sodium azide, pH 7.5	<ul style="list-style-type: none"> • 2–6°C • Protect from light • Avoid freeze-thaw cycles 	When stored undiluted as directed, products are stable for at least 3 months.
F(ab') ₂ fragments	250 µL			For longer storage, divide solution into single-use aliquots and freeze at ≤–20°C, which are stable for at least 6 months.
R-phycoerythrin (R-PE) and allophycocyanin (APC) * conjugates	0.5 mL	1 mg/mL in 0.1 M sodium phosphate, 0.1 M NaCl, 5 mM sodium azide, pH 7.5	<ul style="list-style-type: none"> • 2–6°C • Protect from light • Do not freeze 	When stored undiluted as directed, products are stable for at least 3 months.
Alexa Fluor® dye–R-PE and –APC tandem conjugates	100 µL	1 mg/mL in 0.1 M sodium phosphate, 0.1 M NaCl, 2 mM EDTA, 1% glycerol, 5 mM sodium azide, pH 7.5 †		
BODIPY® FL whole antibody conjugates	1 mg	Lyophilized powder from 0.1 M sodium phosphate, 0.1 M NaCl, 1.5% bovine serum albumin, 0.01% thimerosal, pH 7.5	<ul style="list-style-type: none"> • ≤–20°C • Desiccate • Protect from light • Avoid freeze-thaw cycles 	When stored as directed, products are stable for at least 6 months.
* APC conjugates are prepared from chemically crosslinked APC to avoid dissociation of the molecule into subunits when highly diluted. ¹ † May also contain 1% Prionex reagent as a stabilizer.				
Spectral Data: See Tables 2 and 3.				

Introduction

Invitrogen offers an extensive line of goat anti–rabbit IgG conjugates labeled with a wide selection of premium fluorescent dyes or with biotin (Table 2). We also offer goat anti–rabbit IgG conjugated with fluorescent phycobiliproteins, R-phycoerythrin (R-PE) or allophycocyanin (APC), or with phycobiliprotein–dye “tandem” constructs² (Table 3).

Fluorescent anti–rabbit IgG conjugates are ideal for fluorescence microscopy and confocal laser scanning microscopy, as well as for flow cytometry. In addition to conjugates of whole IgG antibodies, conjugates of F(ab')₂ fragments and highly cross-adsorbed whole antibodies are available in several fluorescent colors. Molecular Probes' strict quality control procedures and

many years of experience in labeling antibodies guarantee that each conjugate provides optimal fluorescence.

In addition to the antibodies listed in this instruction manual, Invitrogen prepares fluorescent conjugates of many other species-specific anti-IgG antibodies, as well as conjugates of avidin, streptavidin, NeutrAvidin™ biotin-binding protein, protein A, and protein G. Refer to www.invitrogen.com or contact Technical Support for more information about these products.

Whole Antibody Conjugates

The goat anti-rabbit whole antibody conjugates are prepared from affinity-purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from rabbit. To minimize cross-reactivity, the goat anti-rabbit whole antibodies have been adsorbed against human IgG, human serum, mouse IgG, and bovine serum. The degree of labeling for each conjugate is typically 2–8 fluorophore or biotin 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 nonspecific staining.

F(ab')₂ Fragment Conjugates

Conjugates of F(ab')₂ fragments are sometimes preferable to whole antibody conjugates for secondary detection, because the absence of the Fc region in F(ab')₂ prevents interactions with Fc receptor-bearing membranes. The F(ab')₂ fragments are prepared from antibodies that have been adsorbed against pooled human serum, mouse serum, mouse plasmacytoma/hybridoma proteins, and purified human paraproteins. The degree of labeling for each conjugate is typically 2–6 fluorophore molecules per F(ab')₂ fragment; the exact degree of labeling is indicated on the product label.

Highly Cross-Adsorbed Whole Antibody Conjugates

For researchers interested in highly cross-adsorbed antibodies, we provide fluorophore-labeled goat anti-rabbit IgG whole antibodies that may be useful in multilabeling experiments or for labeling cells or tissues where nonspecific staining has been a problem. These antibodies have been adsorbed against bovine IgG, goat IgG, mouse IgG, rat IgG, and human IgG. The degree of labeling for each conjugate is typically 2–8 fluorophore or biotin 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 non specific staining.

Guidelines for Use

Preparing BODIPY® FL Conjugates

After reconstitution with 0.5 mL deionized water, the BODIPY® FL product can be stored up to 2 weeks at 2–6°C. For longer storage, divide into single-use aliquots and freeze at ≤–20°C. Frozen aliquots are stable for at least 6 months.

Using Conjugate Solutions

Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant 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 the fluorophore- and biotin-labeled antibodies, including the R-PE-, APC-, and tandem-labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.³

Table 2. Biotin- and fluorescent dye-labeled goat anti-rabbit IgG antibodies.

Label	Ex *	Em *	Whole antibody †	Highly cross-adsorbed ‡	F(ab') ₂ fragment §
Unlabeled	NA	NA	A10533		
Biotin (Nonfluorescent) Conjugates					
Biotin-XX	NA	NA	B2770		B21078
DSB-X™ biotin	NA	NA	D20694	D20695	D20696
Fluorescent Dye Conjugates					
Alexa Fluor® 350	346	442	A11046	A21068	A11069
Marina Blue®	365	460	M10992		
Cascade Blue®	400	420	C2764		
Pacific Orange™	400	551	P31584		
Alexa Fluor® 405	402	421	A31556		
Pacific Blue™	410	455	P10994		
Alexa Fluor® 430	434	539	A11064		
Fluorescein	494	519	F2765		A10526
Alexa Fluor® 488	495	519	A11008	A11034	A11070
Oregon Green® 488	496	524	O6381	O11038	
Alexa Fluor® 500	503	525	A31557		
BODIPY® FL	505	513	B2766		
Oregon Green® 514	511	530	O6384		
Alexa Fluor® 514	518	540	A31558		
Alexa Fluor® 532	531	554	A11009		
Cy3®	552	570	A10520		
Tetramethylrhodamine	555	580	T2769		
Alexa Fluor® 546	556	573	A11010	A11035	A11071
Alexa Fluor® 555	555	565	A21428	A21429	A21430
Rhodamine Red™-X	570	590	R6394		
Alexa Fluor® 568	578	603	A11011	A11036	A21069
Alexa Fluor® 594	590	617	A11012	A11037	A11072
Texas Red®	595	615	T2767		
Texas Red®-X	595	615	T6391		
Alexa Fluor® 610	612	628	A31551		
Alexa Fluor® 633	632	647	A21070	A21071	A21072
Alexa Fluor® 635	633	647	A31576	A31577	
Cy5®	649	670	A10523		
Alexa Fluor® 647 **	650	668	A21244	A21245	A21246
Alexa Fluor® 660 **	663	690	A21073	A21074	A21075
Alexa Fluor® 680 **	679	702	A21076	A21109	A21077
Alexa Fluor® 700 **	702	723	A21038		
Alexa Fluor® 750 **	749	775	A21039		

*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm, for conjugates. Complete spectra for most of these dyes are available at our website (probes.invitrogen.com). † Cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. ‡ Whole antibody, cross-adsorbed against bovine IgG, goat IgG, human IgG, mouse IgG, and rat IgG. § Cross-adsorbed against pooled human serum, mouse serum, mouse plasmacytoma/hybridoma proteins, and purified human paraproteins. ** Human vision is insensitive to light beyond ~650 nm, and therefore it is not possible to view the fluorescence of these dyes by looking through a conventional fluorescence microscope. NA = Not applicable.

Table 3. R-Phycoerythrin, allophycocyanin and tandem conjugates of goat anti-rabbit IgG antibodies.

Label	Ex *	Em *	Cat. no. †	F(ab') ₂ fragment
R-Phycoerythrin (R-PE) and Tandem-R-PE Conjugates				
R-Phycoerythrin	496, 546, 565 ‡	578	P2771	A10542
Alexa Fluor® 610-R-PE	496, 546, 565 ‡	630	A20981	
Alexa Fluor® 647-R-PE	496, 546, 565 ‡	668	A20991	
Alexa Fluor® 680-R-PE	496, 546, 565 ‡	702	A20984	
Allophycocyanin (APC) and Tandem-APC Conjugates				
Allophycocyanin §	650	660	A10931	
Alexa Fluor® 680-APC §	650	702	A21001	
Alexa Fluor® 750-APC §	650	775	A21007	

*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm, for conjugates. † Cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. ‡ Multiple excitation peaks. § Human vision is insensitive to light beyond ~650 nm, and therefore it is not possible to view the fluorescence of these dyes by looking through a conventional fluorescence microscope.

References

1. Cytometry 8, 91 (1987); 2. Our tandem constructs comprise a donor phycobiliprotein, such as R-PE or APC, coupled to a longer-wavelength light-emitting fluorescence acceptor. By the process of fluorescence resonance energy transfer (FRET), an energy transfer cascade is established wherein most of the light absorbed by the donor R-PE or APC results in fluorescence of the acceptor dye. This process can be quite efficient, resulting in almost total transfer of energy to the acceptor dye.; 3. *Short Protocols in Molecular Biology, 2nd Edition*, R.M. Ausubel *et al.*, Eds., John Wiley and Sons (1992) pp. 14-24–14-30.

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

Cat. no.	Product Name	Unit Size
A21068	Alexa Fluor® 350 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11069	Alexa Fluor® 350 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A31556	Alexa Fluor® 405 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11064	Alexa Fluor® 430 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11008	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11034	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11070	Alexa Fluor® 488 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A31557	Alexa Fluor® 500 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A31558	Alexa Fluor® 514 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11009	Alexa Fluor® 532 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11010	Alexa Fluor® 546 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11035	Alexa Fluor® 546 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11071	Alexa Fluor® 546 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A21428	Alexa Fluor® 555 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21429	Alexa Fluor® 555 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21430	Alexa Fluor® 555 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A11011	Alexa Fluor® 568 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11036	Alexa Fluor® 568 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21069	Alexa Fluor® 568 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A11012	Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11037	Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11072	Alexa Fluor® 594 goat anti-rabbit IgG (H+L), F(ab') ₂ fragment *2 mg/mL*	250 µL
A31551	Alexa Fluor® 610 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL

A20981	Alexa Fluor® 610–R-phycoerythrin goat anti-rabbit IgG (H+L) *1 mg/mL*	100 µL
A21070	Alexa Fluor® 633 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21071	Alexa Fluor® 633 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21072	Alexa Fluor® 633 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A31576	Alexa Fluor® 635 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A31577	Alexa Fluor® 635 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21244	Alexa Fluor® 647 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21245	Alexa Fluor® 647 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21246	Alexa Fluor® 647 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A20991	Alexa Fluor® 647–R-phycoerythrin goat anti-rabbit IgG (H+L) *1 mg/mL*	100 µL
A21001	Alexa Fluor® 680–allophycocyanin goat anti-rabbit IgG (H+L) *1 mg/mL*	100 µL
A21073	Alexa Fluor® 660 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21074	Alexa Fluor® 660 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21076	Alexa Fluor® 680 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21109	Alexa Fluor® 680 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21075	Alexa Fluor® 660 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A21077	Alexa Fluor® 680 F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A20984	Alexa Fluor® 680–R-phycoerythrin goat anti-rabbit IgG (H+L) conjugate *1 mg/mL*	100 µL
A21038	Alexa Fluor® 700 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21039	Alexa Fluor® 750 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21007	Alexa Fluor® 750–allophycocyanin goat anti-rabbit IgG (H+L) *1 mg/mL*	100 µL
A10931	allophycocyanin, crosslinked, goat anti-rabbit IgG (H+L) *1 mg/mL*	0.5 mL
B2770	biotin-XX goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
B21078	biotin-XX F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
B2766	BODIPY® FL goat anti-rabbit IgG (H+L)	1 mg
C2764	Cascade Blue® goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A10520	Cy3® goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A10523	Cy5® goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
D20694	DSB-X™ biotin goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
D20695	DSB-X™ biotin goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
D20696	DSB-X™ biotin F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
A10526	fluorescein F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *2 mg/mL*	250 µL
F2765	fluorescein goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A10533	goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
G21080	goat anti-rabbit IgG (H+L), CMNB-caged fluorescein conjugate *2 mg/mL*	250 µL
M10992	Marina Blue® goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
O6381	Oregon Green® 488 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
O11038	Oregon Green® 488 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
O6384	Oregon Green® 514 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
P10994	Pacific Blue™ goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
P31584	Pacific Orange™ goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
P2771	R-phycoerythrin goat anti-rabbit IgG (H+L) *1 mg/mL*	0.5 mL
A10542	R-phycoerythrin F(ab') ₂ fragment of goat anti-rabbit IgG (H+L) *1 mg/mL*	250 µL
R6394	Rhodamine Red™-X goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
T2769	tetramethylrhodamine goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
T2767	Texas Red® goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
T6391	Texas Red®-X goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL

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