Revised: 03–July–2001

Labeled Rabbit Anti–Mouse IgG Antibodies

Quick Facts

Storage upon receipt:
- 4°C
- Protect from light

Abs/Em: See Table 1

Working concentrations: 1–10 µg/mL

Introduction

Molecular Probes' fluorescent rabbit anti–mouse IgG antibodies (Table 1) are prepared from affinity-purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from mouse. To minimize cross-reactivity, the rabbit anti–mouse IgG antibodies have been adsorbed against human serum proteins prior to labeling. Conjugates of $F(ab')_2$ fragments are sometimes preferable to whole antibody conjugates for secondary detection, since the absence of the Fc region in $F(ab')_2$ fragments prevents interactions with Fc receptor–bearing membranes. Please note: the rabbit Fc region will bind nonspecifically to human tissue. Molecular Probes recommends that customers use the $F(ab')_2$ fragment when using the antibodies on human tissues. The Alexa Fluor® dyes to which many of these antibodies are conjugated provide for extraordinarily bright antibody conjugates. The approximate absorption and fluorescence emission maxima for each of the conjugates are shown in Table 1.

In addition to the secondary antibodies described in this Product Information sheet, Molecular Probes 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. Please consult our Web site at www.probes.com or contact our Technical Assistance Department for more information about these products.

Materials

Contents

Fluorophore-Labeled Antibodies

The fluorophore-labeled rabbit anti–mouse IgG (H+L) antibodies are supplied in unit sizes of 0.5 mL as 2 mg/mL solutions in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide.

Table 1. *Molecular Probes' labeled rabbit anti–mouse IgG antibodies.*

Label	Abs *	Em *	Rabbit anti–mouse IgG †	Rabbit anti–mouse IgG, $F(ab')_2$ †
Biotin-XX	NA	NA	B-21066	
Alexa Fluor 350	346	442	A-21062	
Alexa Fluor 488	495	519	A-11059	A-21204
Alexa Fluor 546	556	573	A-11060	
Alexa Fluor 555	555	565	A-21427	
Alexa Fluor 568	578	603	A-11061	
Alexa Fluor 594	590	617	A-11062	A-21205
Texas Red-X	595	615	T-21067	
Alexa Fluor 633	632	647	A-21063	
Allophycocyanin	650	660	A-10930	
Alexa Fluor 647	650	668	A-21239	
Alexa Fluor 660	663	690	A-21064	
Alexa Fluor 680	679	702	A-21065	

***** Approximate absorption (Abs) and fluorescence emission (Em) maxima in nm. Complete spectra for these dyes are available at our Web site (www.probes.com). NA, not applicable. † These rabbit anti–mouse IgG antibodies have been adsorbed against human serum proteins to minimize crossreactivity.

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.

Allophycocyanin-Labeled Antibodies

The allophycocyanin rabbit anti–mouse IgG antibody conjugate (A-10930) is supplied in a unit size of 0.5 mL of a 1 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 2 mM sodium azide. Molecular Probes' allophycocyanin conjugates are prepared from chemically crosslinked allophycocyanin to avoid dissociation of the molecule into subunits when highly diluted.[1]

Fluorophore- and Biotin-Labeled $F(ab')_2$ Fragments

Conjugates of $F(ab')_2$ fragments are provided in unit sizes of 250 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 2–6 fluorophore or biotin molecules per $F(ab')_2$ fragment; the exact degree of labeling is indicated on the product label.

Storage

When these products are stored undiluted at 4°C and protected from light, 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 FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

Application

It is a good practice to centrifuge the protein conjugate solution 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.[2] For the allophycocyanin-labeled antibodies 1–3 µg of conjugate is usually sufficient to label ~1 million cells.

References

1. Cytometry 8, 91 (1987); **2.** *Short Protocols in Molecular Biology, 2nd Edition*, F.M. Ausubel et al., Eds., John Wiley and Sons (1992) pp. 14-24–14-30.

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

Cat #	Product Name	Unit Size
A-10930	allophycocyanin, crosslinked, rabbit anti-mouse IgG (H+L) *1 mg/mL*	0.5 mL
A-21062	Alexa Fluor® 350 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-11059	Alexa Fluor® 488 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-21204	Alexa Fluor® 488 $F(ab')_2$ fragment of rabbit anti-mouse IgG (H+L) *2 mg/mL*	250 µL
A-11060	Alexa Fluor® 546 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-21427	Alexa Fluor® 555 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-11061	Alexa Fluor® 568 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-11062	Alexa Fluor® 594 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-21205	Alexa Fluor® 594 $F(ab')_2$ fragment of rabbit anti-mouse IgG (H+L) *2 mg/mL*	250 µL
A-21063	Alexa Fluor® 633 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-21239	Alexa Fluor® 647 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-21064	Alexa Fluor® 660 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A-21065	Alexa Fluor® 680 rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
B-21066	biotin-XX rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
T-21067	Texas Red®-X rabbit anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL

Contact Information

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.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

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