

Labeled Donkey Anti–Rabbit IgG Antibodies

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
Fluorophore-labeled donkey anti-rabbit IgG (H+L) antibodies	0.5 mL	2 mg/mL solutions in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, 5 mM sodium azide	<ul style="list-style-type: none"> • 2–8°C • Protect from light • Avoid freeze-thaw cycles 	When stored as directed, products are stable for at least 3 months.
Degree of labeling: Typically 2–8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.				
Approximate fluorescence excitation/emission maxima: See Table 2, page 2.				

Introduction

Fluorescent donkey anti–rabbit IgG antibodies (Table 2, page 2) are prepared from affinity-purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from rabbit. The Alexa Fluor® dyes to which these antibodies are conjugated provide for extraordinarily bright antibody conjugates. The donkey anti–rabbit IgG antibodies show minimum crossreactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rat, and sheep serum proteins. The approximate fluorescence excitation and emission maxima for each of the conjugates are shown in Table 2, page 2. For Qdot® nanocrystal conjugates of donkey anti–rabbit IgG antibodies, see Table 3, page 2.

In addition to the secondary antibodies described in this manual, Molecular Probes offers 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. For more information about these products, refer to our website at www.lifetechnologies.com or contact Technical Support.

At the time of preparation, the products are certified to be free of unconjugated dyes and are tested in an immunofluorescence experiment to ensure low non-specific staining.

Table 2 Labeled donkey anti-rabbit IgG antibodies*

Catalog no.	Label	Ex †	Em †
A10039	Alexa Fluor® 350	346	442
A21206	Alexa Fluor® 488	495	519
A10040	Alexa Fluor® 546	556	573
A31572	Alexa Fluor® 555	555	565
A10042	Alexa Fluor® 568	578	603
A21207	Alexa Fluor® 594	590	617
A31573	Alexa Fluor® 647‡	650	668
A10043	Alexa Fluor® 680‡	663	690
A11374	Alexa Fluor® 790‡	784	814

* Minimum crossreactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, mouse, rat, and sheep serum proteins. † Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm, for conjugates. ‡ 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.

Table 3 Qdot® conjugates of donkey anti-rabbit IgG antibodies

Catalog no.	Label	Ex *	Em *
Q22074	Qdot® 525	<525	525
Q22077	Qdot® 565	<565	565
Q22080	Qdot® 585	<585	585
Q22083	Qdot® 605	<605	605
Q22086	Qdot® 625	<625	625
Q22089	Qdot® 655	<655	655

* Qdots are excitable (Ex, in nm) at any wavelength below their emission maxima (Em, in nm). For most practical applications, they should be excited at wavelengths below 450 nm. For additional information, refer to the product manual "Qdot® Streptavidin Conjugates", available at www.lifetechnologies.com/manuals.

Guidelines for Use

We recommend centrifuging the protein conjugate solution briefly in a microcentrifuge before use and add only the supernatant to the experiment. This step eliminates any protein aggregates that may have formed during storage, thereby reducing non-specific 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

Alexa Fluor® 680 and Alexa Fluor® 790 IgG conjugates have been validated for fluorescent western detection and are compatible with most standard fluorescent western instrumentation. They are amenable to standard western blot fluorescent detection protocols and can be used in single or multicolored experiments. For more information, refer to the technical note: Multicolored western detection using Alexa Fluor® secondary antibodies, available for downloading at www.lifetechnologies.com or by contacting Technical Support.

Reference

1. *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 website or from our Customer Service Department.

Cat. no	Product Name	Unit Size
A10039	Alexa Fluor® 350 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21206	Alexa Fluor® 488 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A10040	Alexa Fluor® 546 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A31572	Alexa Fluor® 555 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A10042	Alexa Fluor® 568 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21207	Alexa Fluor® 594 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A31573	Alexa Fluor® 647 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A10043	Alexa Fluor® 680 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11374	Alexa Fluor® 790 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL

Visit www.lifetechnologies.com/antibody for details on antibody research reagents and tools.

Purchaser Notification

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These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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