Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 555
Excitation/Emission Max	553/568 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_162543

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	4 µg/mL	0 Publication
Flow Cytometry (Flow)	Assay-dependent	0 Publication
Functional Assay (Functional)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

These donkey anti-rabbit IgG (H+L)whole secondary antibodies have been affinity-purified and show minimum cross-reactivity. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there may be the presence of endogenous immunoglobulins.

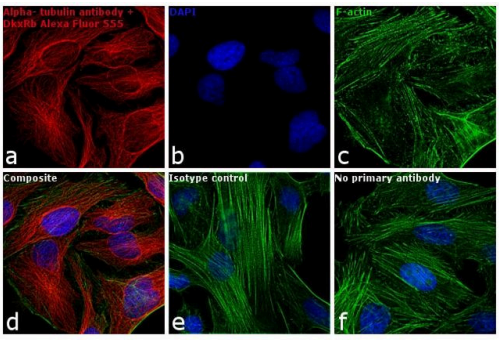
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and

high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 555 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. 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 certificate of analysis for each product lot.

Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help 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-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

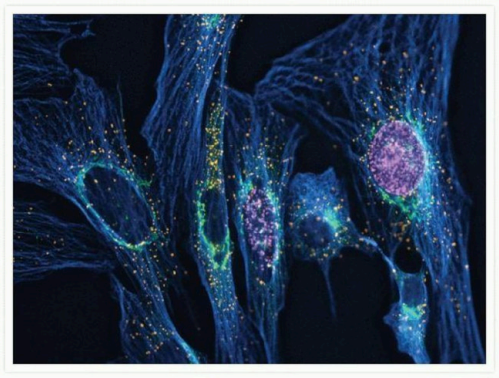
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555



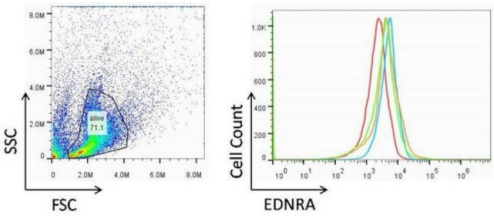
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31572) in ICC/IF

Immunofluorescence analysis of Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 555 conjugate was performed using HeLa cells stained with alpha Tubulin Rabbit Polyclonal Antibody (Product # PA516891) The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 µg/mL primary antibody for 3 hours at room temperature. Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 555 conjugate (Product # A-31572) was used at a concentration of 4 µg/mL in phosphate buffered saline containing 0.2% BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379), 1:300) (Panel c: green). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31572) in ICC/IF

Muntjac cells were treated with 10 µM EdU (Product # A10044) for 45 min; cells were then fixed and permeabilized, and EdU that had been incorporated into newly synthesized DNA was detected using the far red-fluorescent Click-iT™ EdU Alexa Fluor® 647 High-Throughput (HCS) Assay (Product # A10208), utilizing the technical tip for converting the HCS assay to conventional fluorescence microscopy. Tubulin was labeled with an anti-alpha-tubulin antibody (Product # A11126) and visualized with Alexa Fluor® 350 Goat Anti-Mouse IgG (Product # A-11045, A21049). The Golgi complex was stained with green-fluorescent Alexa Fluor® 488 conjugate of lectin HPA from Helix pomatia (edible snail) (Product # L11271), and peroxisomes were labeled with an anti-peroxisome antibody and visualized with orange-fluorescent Alexa Fluor® 555 Donkey Anti-Rabbit IgG (Product # A-31572).



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31572) in Flow

Flow cytometric analysis of EDNRA on mouse bone marrow cells. Cells were harvested, fixed with 2% formalin for 15 minutes at room temperature, washed with 2% BSA in PBS, and incubated with an EDNRA polyclonal antibody (Product # PA5-13432) at dilutions of 1:10 (orange histogram), 1:50 (blue histogram), and 1:100 (green histogram), or a rabbit IgG isotype control (red histogram) for 30 minutes at room temperature. Following the primary antibody incubation, cells were washed, and then incubated with an Alexa Fluor 555-conjugated anti-rabbit IgG secondary antibody (Product # A-31572) for 30 minutes at room temperature. Cells were washed again, and the same number of events per sample was acquired on a flow cytometer. Data courtesy of the Innovators Program.

View more figures on thermofisher.cn

2415 References

Canonical and non-canonical PRC1 differentially contribute to regulation of neural stem cell fate. Life Sci Alliance (2025)

Revealing the biological features of the axolotl pancreas as a new research model. Front Cell Dev Biol (2025)

Genetic regulation of TERT splicing affects cancer risk by altering cellular longevity and replicative potential. Nat Commun (2025)

Sox9 inhibits Activin A to promote biliary maturation and branching morphogenesis. Nat Commun (2025)

Identification of Meibomian gland stem cell populations and mechanisms of aging. Nat Commun (2025)

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