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 (FN)	-	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.

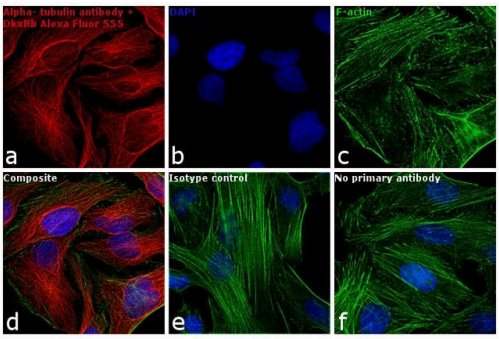
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive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

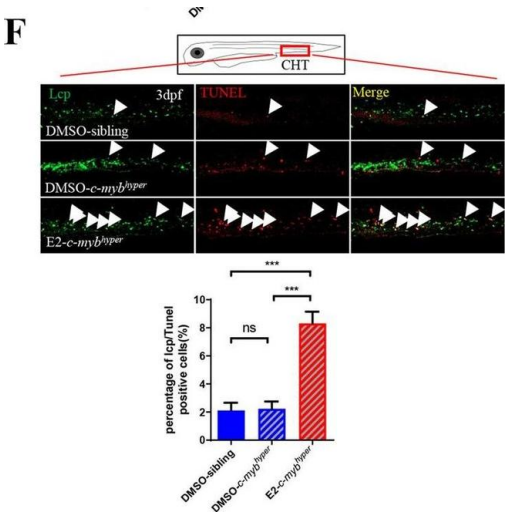
Product will be shipped at Room Temperature.

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



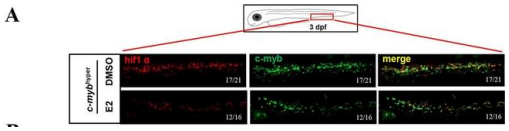
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31572) in ICC/IF

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

E2 decreases the number of neutrophils in c-mybhyper zebrafish, mainly through inhibiting cell proliferation and promoting cell apoptosis.A E2 exposure decreased SB positive cells in the CHT region. (t-test, ***p < 0.001, **p < 0.01. n > 20). B E2 exposure decreased lyz in the CHT region, as determined by WISH. (t-test, ***p < 0.001, **p < 0.01. n > 20). C The qPCR quantification of the decrease in lyz expression with E2 (t-test, mean ± SEM; ***p < 0.001, **p < 0.01. n 10). D May-Grunwald-Giemsa staining of whole KM blood cells in 6-month-old c-mybhyper animals followed by four days of E2 treatment (t-test, ***p < 0.001. n = 12). Red arrowheads, blue asterisks, black arrowheads and yellow lightning indicate neutrophils, precursors, lymphocytes and macrophages, respectively. E Double staining of bromodeoxyuridine (BrdU)/Lcp indicated decreased neutrophil proliferation in c-mybhyper zebrafish embryos treated with E2. (one-way ANOVA (LSD) ***p < 0.001, n = 12). F The TUNEL assays showed the effect of E2 on the apoptosis of myeloid lineage in zebrafish embryos (one-way ANOVA (LSD) ***p < 0.001, ns, no significance. n = 12). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35842445), licensed under a CC BY license.



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

Hif1 participates in E2 induced down-regulation of c-myb and neutrophil hyperplasia.A Double staining of hif1 and c-myb-GFP with antibodies with or without E2 treatment. B Knockdown (MO) or inhibition (PX-478) of hif1 decreased c-myb and lyz expression by WISH as well as SB positive neutrophils in c-mybhyper zebrafish. (t-test, ***p < 0.001, **p < 0.01, *p < 0.05, n > 20). C Overexpression of hif1 reversed E2 induced down-regulation of c-myb and lyz expression by WISH and neutrophil hyperplasia by SB staining. (t-test, ***p < 0.001, **p < 0.01, *p < 0.05, n > 20). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35842445), licensed under a CC BY license.

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2044 References

Maternal exposure to hyperbaric oxygen at the preimplantation stages increases apoptosis and ectopic Cdx2 expression and decreases Oct4 expression in mouse blastocysts via Nrf2-Notch1 upregulation and Nf2 downregulation. Dev Dyn (2024)

Tumor necrosis factor-stimulated gene-6 ameliorates early brain injury after subarachnoid hemorrhage by suppressing NLRC4 inflammasome-mediated astrocyte pyroptosis. Neural Regen Res (2024)

SARS-CoV-2 spike protein induces endothelial dysfunction in 3D engineered vascular networks. J Biomed Mater Res A (2024)

A role for TGF signaling in Gli1+ tendon and enthesis cells. FASEB J (2024)

PCSK9 stimulates Syk, PKC, and NF-B, leading to atherosclerosis progression independently of LDL receptor. Nat Commun (2024)