



Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546

Product Details	
Size	1 mg
Species Reactivity	Goat
Host/Isotype	Donkey / IgG
Class	Polyclonal
Туре	Secondary Antibody
Conjugate	Alexa Fluor™ 546
Excitation/Emission Max	561/572 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_2534103

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 μg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 μg/mL	0 Publication
Flow Cytometry (Flow)	1-10 μg/mL	-
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

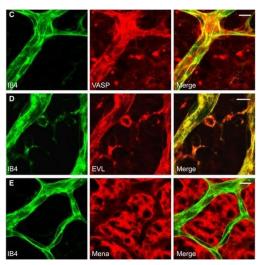
To minimize cross-reactivity, these donkey anti-goat IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against rabbit, rat, mouse, and human IgG. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity 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 are may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 546 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 546 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 546 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 546 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

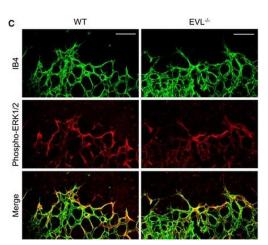
exact degree of labeling is indicated on the certificate of analysis for each product lot.

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 μ g/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

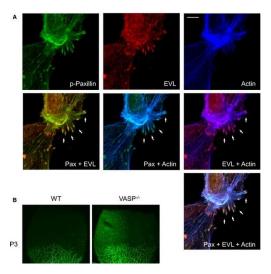
Product will be shipped at Room Temperature.



Goat IqG (H+L) Cross-Adsorbed Secondary Antibody (A-11056) in ICC/IF Expression of Ena/VASP proteins in postnatal retinal endothelial cellsA, BRNA sequencing of CD31 and CD34 double-positive retinal endothelial cells from P5 wild-type mice. (A) RNA levels (FPKM; fragments per kilobase million) of marker genes of endothelial cells (green; CD31 (gene name PECAM1), CD34, von Willebrand Factor (vWF), tyrosine-protein kinase receptor Tie2 (TEK), VEcadherin (CDH5), endoglin (ENG), CD146 (MCAM) and VEGFR2 (KDR)), astrocytes (blue, GFAP), immune cells (red; T cells (CD3E), B-cells (CD19), all leukocytes (CD45, PTPRC), and monocytes/macrophages (F4/80, EMR1)), Müller glial cells (orange; aquaporin 4 (AQP4)), neurons (yellow; retinal ganglion cells (RNA binding fox-1 homolog 3, RBFOX3), amacrine cells (parvalbumin, PVALB), bipolar cells (PKC-, PRKCA), horizontal cell (calbindin, CALB1), photoreceptors (rods, CD73 (NT5E); cones, transducing (GNAT1)), and retinal pigment epithelial cells (magenta; retinal pigment epithelium-specific 65kDa protein (RPE65). (B) RNA levels of VASP (red), EVL (green), and Mena (blue) in the CD31 and CD34 double-positive P5 retinal endothelial cells. Error bars represent SEM; n=18 animals (36 retinas) from six independent litters; four independent experiments. ***P<0.001, one-way ANOVA with Bonferroni's multi comparison test.C-EStaining of Ena/VASP proteins in blood vessels of P5 mouse retinas. P5 wild-type mouse retinas were fixed and stained with i... Image collected and cropped by CiteAb from the following publication (https://pubmed. ncbi.nlm.nih.gov/33512764), licensed under a CC BY license.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11056) in ICC/IF EVL deficiency impairs VEGFR2 signalingWestern blots and quantification of VEGFR2 phosphorylation levels relative to total VEGFR2 levels in WT and EVL-/endothelial cells under basal and VEGF-stimulated conditions (80ng/mL); n=5 independent cell batches, error bars represent SEM; *P<0.05, one-way ANOVA with Bonferroni's multi comparison test. Western blots and quantification of ERK1 /2 phosphorylation levels relative to total ERK levels in WT and EVL-/endothelial cells under basal and VEGF-stimulated conditions (80ng/mL); n=6 independent cell batches, error bars represent SEM; *P<0.05, one-way ANOVA with Bonferroni's multi comparison test. ERK1/2 phosphorylation in P5 retinas of wild-type (WT) and global EVL-/- mice. Retinas were fixed and stained with isolectin B4 (IB4, green) to visualize endothelial cells and antibodies directed against phospho-ERK1/2 (red). Representative images from three independent experiments are shown. Scale bars, 100µm. Analysis of endothelial ERK1/2 phosphorylation normalized to wild-type littermates. Error bars represent SEM; ***P<0.001, unpaired Student's t-test; two different litters. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov /33512764), licensed under a CC BY license.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11056) in ICC/IF Subcellular localization of EVL and postnatal angiogenesis in VASP-/- miceAEVL localizes to focal adhesions in endothelial cells. MLEC from wild-type mice were stained for phospho-paxilin (green) as a marker for focal adhesions, EVL (red) and actin (blue). White arrows indicate integrin-based focal adhesions at the tips of actin stress fibers. Representative images from three independent experiments are shown. Scale bar, 10µm.B, CPostnatal retinal angiogenesis in VASP-/- mice. (B) Isolectin B4-stained vasculature in whole mount retinas of wildtype (WT) and global VASP-/- mice on postnatal days 3 and 5 (P3, P5) assessed by confocal microscopy. Scale bars 200µm. (C) Analysis of the radial vascular outgrowth relative to retinal radius and normalized to wild-type littermates. Error bars represent SEM; no significant difference was observed between the two genotypes at P3 (P>0.999) or P5 (P>0.999) (one-way ANOVA with Bonferroni's multi-comparison test). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33512764), licensed under a CC BY license.

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□ 466 References

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