Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 647
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_2536183

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 µg/mL	-
Immunohistochemistry (Frozen) (IHC (F))	1:1,000	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-

Product Specific Information

To minimize cross-reactivity, these donkey anti-rabbit IgG whole antibodies have been affinity-purified and show a published cross-reactivity to rat 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 may be the presence of endogenous immunoglobulins.

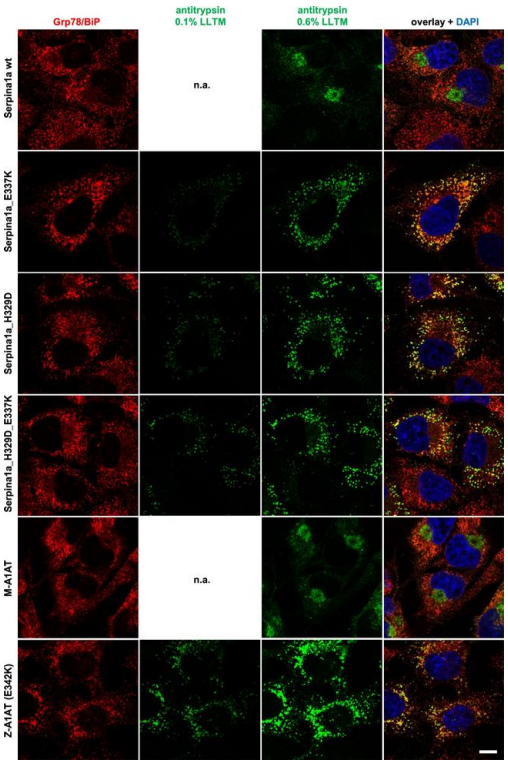
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 647 dye is a near-infrared-fluorescent dye with excitation ideally suited to the 647 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 647 dye is pH-insensitive over 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 647 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.

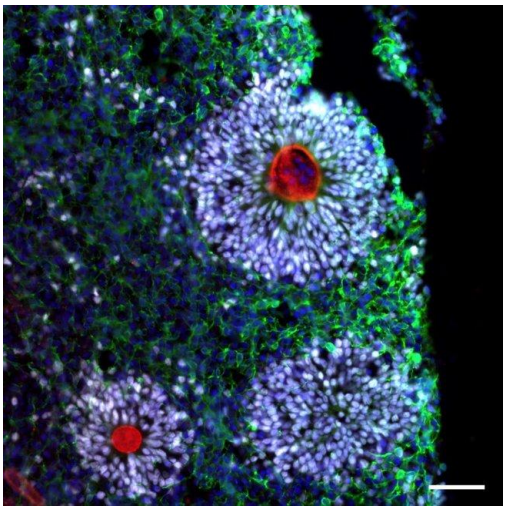
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647



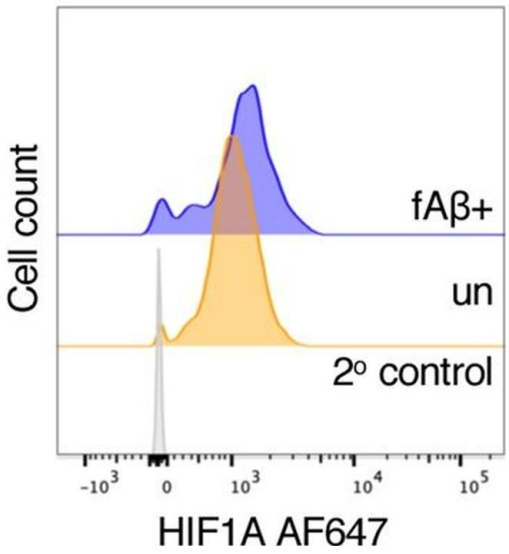
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31573) in ICC /IF

Serpina1a mutants imitate intracellular distribution of human Z-A1AT. Confocal laser immunofluorescence analysis of COS-7 cells expressing wild type (wt; top row), E337K, H329D or H329D_E337K double mutant Serpina1a (second to fourth row), compared to cells overexpressing normal human M-A1AT or E342K Z-A1AT (fifth and sixth row). Mouse Serpina1a and human A1AT were stained with Alexa Fluor 568 secondary antibody (green) and exposed to 0.6% laser light transmission (LLTM). Mutant-expressing cells were additionally exposed to 0.1% LLTM, as the very strong signal resulted in over-saturation at 0.6%. ER-marker Grp78/BiP was stained with Alexa Fluor 647 secondary antibody (red) and cell nucleus was stained using DAPI (blue). Scale bar: 10 µm. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/s41598-019-44043-3), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31573) in IHC (F)

Immunofluorescent analysis of ZO-1 (red) and SOX2 (grey) in human iPSC-derived forebrain organoids derived at Day 40. The organoids were fixed with 4% PFA for 1 hour at room temperature, followed by incubation with 30% sucrose solution overnight at 4°C. The organoids were then embedded in OCT and cryosectioned at 5 µm, permeabilized with 0.2% Triton X-100 for 20 min, and blocked with 10% donkey serum in PBS for 30 min at room temperature. Organoid slices were stained with a Mouse ZO-1 monoclonal antibody (red; Product # 33-9100) at a dilution of 1:500, a Rabbit SOX2 polyclonal antibody (grey; Product # PA1-094X) at a dilution of 1:500, and a Chicken MAP2 polyclonal antibody (green) at a dilution of 1:1000 in blocking buffer overnight at 4°C, and then incubated with Donkey anti-Mouse Alexa Fluor 568 (Product # A10037), Donkey anti-Rabbit Alexa Fluor 647 (Product # A31573), and Donkey Anti-Chicken Alexa Fluor 488 at a dilution of 1:1000 as well as DAPI (blue; 1:25000) in blocking solution at room temperature for 1 hour. Images were taken at 20X magnification. Scale bar: 50 µm. Data courtesy of Dr. Zhexing Wen at Emory University.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31573) in Flow
The gene expression signature associated with XO4+ microglia is molecularly and functionally replicated in microglia isolated from the brains of AD patients and non-AD patients.a–c UMAP projection of single microglia nuclei from control and AD patient entorhinal and frontal cortex samples, combined by integrating data from51–54, comprising 102 patients; AD (n = 5891 microglia nuclei), mild AD (n = 1591 microglia nuclei), controls (n = 2988 microglia nuclei), Other Dementia (n = 3 microglia nuclei) and TREM2 R62H variant (n = 1458 microglia nuclei). Clustering and analysis of signature scores is performed using Seurat v3. UMAP projection is coloured by (a) study of origin, (b) Seurat cluster and (c) XO4+ score. d Box plots for gene signature scores in each human microglial cluster for the AD vs Trem2KO AD signature, AD vs WT signature51, DAM vs homeostatic, and DAM2 vs DAM1 signatures13. The lower, middle and upper hinges represent the lower quartile, median and upper quartile, respectively, while the upper and lower whiskers extend ±1.5 times of the interquartile range from the hinges. For each signature score category, pairwise Wilcoxon test between each cluster and base mean was computed. Multiple testing was corrected for using Bonferroni correction. *p < 0.05, **p < 0.01; ***p < 0.001, ****p < 0.0001, exact p values are provided in the Source data. e The proportion of cells in Clusters … Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-021-23111-1), licensed under a CC BY license.

1341 References

Directed differentiation of human pluripotent stem cells to epicardial-derived fibroblasts. STAR Protoc (2022)

A Plasmodium falciparum ATP-binding cassette transporter is essential for liver stage entry into schizogony. iScience (2022)

SUMO1 Modification of Tau in Progressive Supranuclear Palsy. Mol Neurobiol (2022)

Activation of Xist by an evolutionarily conserved function of KDM5C demethylase. Nat Commun (2022)

Apical-out airway organoids as a platform for studying viral infections and screening for antiviral drugs. Sci Rep (2022)