Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 594
Excitation/Emission Max	590/618 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_141637

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	1:500	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	2 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
in situ PLA (PLA)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

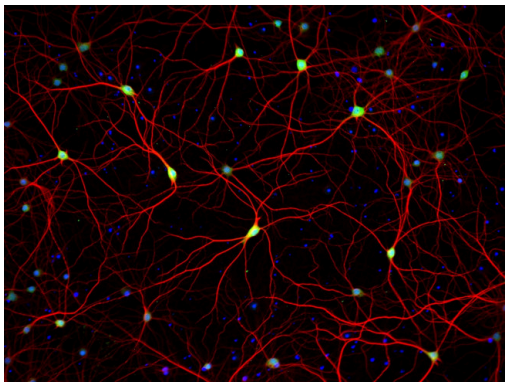
To minimize cross-reactivity, these donkey anti-rabbit IgG whole antibodies have been affinity-purified and show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rat, and sheep serum proteins. Cross-adsorption or pre-adsorption is a purification step to increase 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 594 dye is a bright, red-fluorescent dye with excitation ideally suited to the 594 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 594 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 594 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.

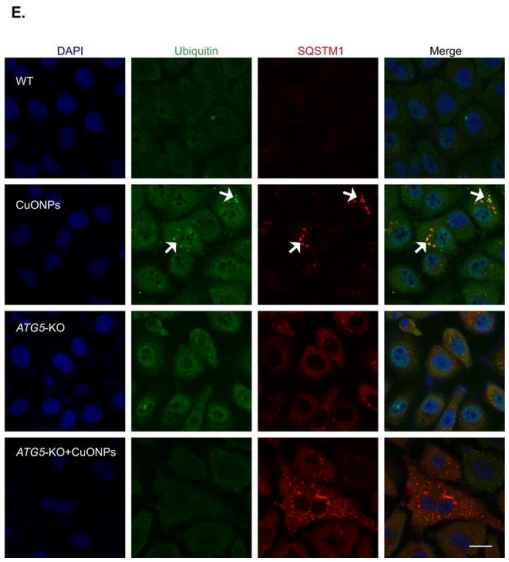
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594



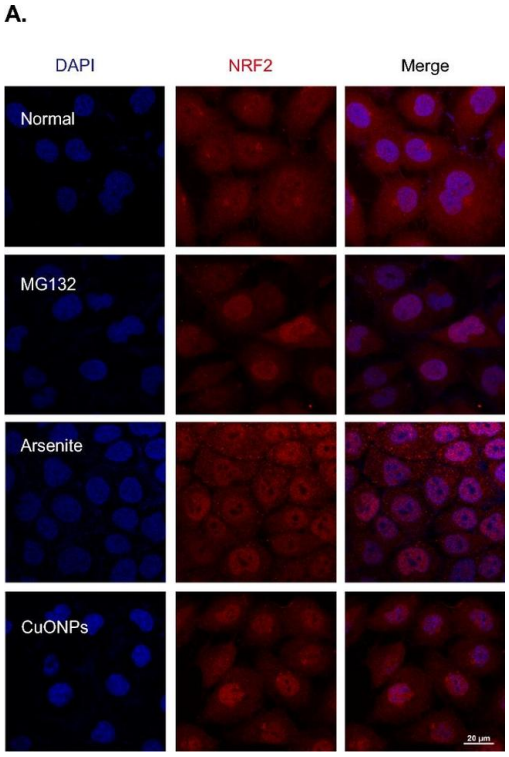
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21207) in ICC/IF

Immunofluorescent analysis of HuC/D (green) and MAP2 (red) on rat primary cortical neurons cultured for 28 days in the B-27 Plus Neuronal Culture System (Product # A3653401). At day 28 the cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.1% triton x-100 for 30min, and blocked with 1% BSA for 30 min at room temperature. Cells were stained with anti-HuC/D antibody (Product # A-21271) at a dilution of 1:250, and anti-MAP2 (Product # PA5-17646) at a dilution of 1:250, in 1% BSA staining buffer, overnight at 4C, and then incubated with Alexa Fluor 488 conjugated donkey anti-mouse (Product # A-21202) and Alexa Fluor 594 donkey anti-rabbit (Product # A-21207) antibodies at a dilution of 1:1000 for 30 min. at room temp. Wash 3 times with DPBS. Stain with DAPI for nucleus. Images were taken on a Thermo Fisher Scientific EVOS M5000 Cell Imaging System at 10x magnification.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21207) in ICC/IF

Autophagy inhibition activates ubiquitin-proteasome pathway in CuONPs-treated cells. A and C Immunoblotting analysis and quantification of ubiquitinated proteins levels in HUVECs treated with 0, 5, 10, 15 and 20 μg/ml CuONPs for 12 h. GAPDH was used as loading control. B and D Immunoblotting analysis and quantification of ubiquitinated proteins levels in HUVECs treated with CuONPs (20 μg/ml) for 0, 3, 6, 9 and 12 h, respectively. GAPDH was used as loading control. E Representative confocal images of WT and ATG5-KO cells treated with CuONPs (20 μg/ml), respectively. The cells were immunofluorescently stained and analyzed with ubiquitin and SQSTM1 antibody. F and G Immunoblotting analysis and quantification of the levels of NRF2, HMOX1, ubiquitinated proteins, SQSTM1, LC3B and GAPDH (loading control) in WT and ATG5-KO cells treated with 0, 5, 10, 15, 20 and 30 μg/ml CuONPs for 12 h, respectively. H and I Immunoblotting analysis and quantification of NRF2 half-life in CuONPs-treated HUVECs. Cells were treated with tBHQ (10 μM) and CuONPs (20 μg/ml) for 9 h, and then treated with CHX (50 μg/ml) for 0, 1, 2, 3, 6, 9 h, respectively. -Actin served as loading control. H Immunoblotting analysis and quantification of NRF2 half-life in CuONPs-treated WT or ATG5-KO HUVECs cells. Cells were treated with tBHQ (10 μM) and CuONPs (20 μg/ml) for 9 h, and then treated with CHX (50 … Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35690781), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21207) in ICC/IF

The activation of NRF2 in CuONPs-treated HUVECs. A Representative confocal images of NRF2 in HUVECs cells treated with CuONPs (20 μg/mL) for 12 h. Scale bar, 20 μm. Nuclei were stained with DAPI. MG132 or arsenite were used as positive controls. B, C Immunoblotting analysis and quantification of protein levels of NRF2 and its downstream HMOX1 and GCLM in HUVECs cells treated with 0, 5, 10, 15 and 20 μg/mL CuONPs for 12 h, respectively. GAPDH served as the internal control. D, E Immunoblotting analysis and quantification of protein levels of NRF2, HMOX1 and GCLM in HUVECs cells treated with 20 μg/mL CuONPs for 0, 3, 6, 9 and 12 h, respectively. GAPDH served as the internal control. F qPCR analysis of the mRNA levels of HMOX1, GCLM, SLC7A11, NQO1 and TXN in wild-type (WT) or NRF2 knockout (NRF2-KO) cells treated with 20 μg/mL CuONPs for 0, 6 and 9 h, respectively. G, H Immunoblotting analysis and quantification of NRF2 and HMOX1 protein levels in WT or NRF2-KO cells treated with 20 μg/mL CuONPs for 0, 6 and 9 h, respectively. -Actin was used as loading control. All data are representative of three independent experiments. In C and E, Student's t-test was used for statistical analysis. In F and H, one-way ANOVA followed by a Tukey multiple comparison test was used for statistical analysis. The values are expressed in mean ± S.D. ns not significance; **", P 0.01; &… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35690781), licensed under a CC BY license.

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2441 References

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