Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

Product Details

Size: 100µl
Species Reactivity: Rat
Host/Isotype: Donkey / IgG
Class: Polyclonal
Type: Secondary Antibody
Conjugate: Alexa Fluor™ 488
Excitation/Emission Max: 499/520 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: BA2005

Applications

Immunohistochemistry (IHC): 1-10 µg/mL
Immunocytochemistry (ICC/IF): 1 µg/mL

Product Specific Information

These donkey anti-rat IgG (H+L)whole secondary antibodies have been affinity-purified and show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rabbit, 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.

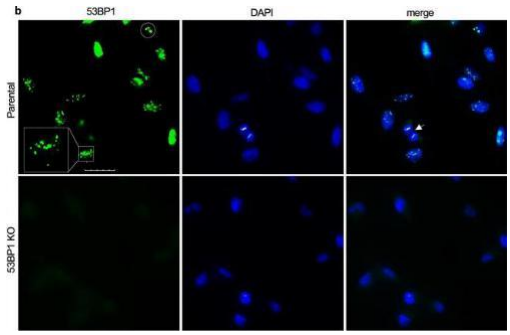
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over 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 488 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

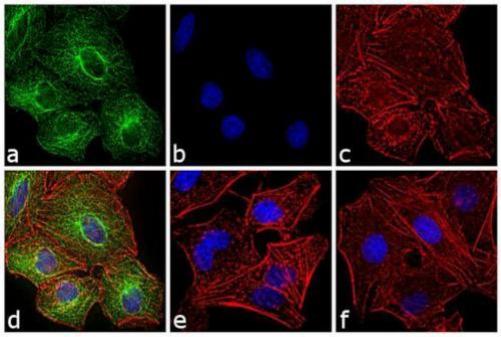
thesupernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the 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.

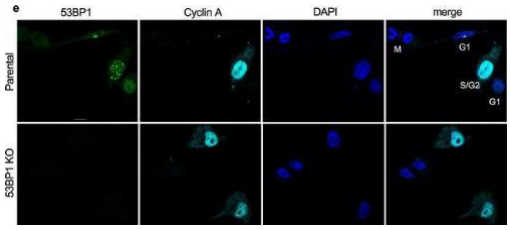
Product Images For Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21208) in ICC/IF 53BP1 forms nuclear puncta.a MDA-MB-231 parental and 53BP1 knockout (KO) cells were treated or not with 500 nM CPT for 6 h, and protein expression was detected. Arrowhead indicates 53BP1. b Representative images of nuclear localization of 53BP1 in MDA-MB-231 cells under normal growth conditions. Single z-plane images were acquired by sequential scanning using confocal microscopy. Square: an example of puncta+ cell; Circle: a cell with three spontaneous 53BP1 foci; Arrow: a mitotic cell. Scale bar is 16 µm. c Percentage of puncta positive cells were analyzed from n = 36 individual images taken from four replicate experiments with a total n = 447 and 373 cells analyzed for parental and KO groups, respectively. Data represent mean values and standard deviation (SD). d Violin plot of 53BP1 puncta number per cell was analyzed from n = 185 and 105 parental and 53BP1 KO MDA-MB-231 cells, respectively. Data represent mean, 25th, and 75th percentiles with the whiskers extending to the minimum and maximum values. e Representative images of 53BP1 puncta and Cyclin A in MDA-MB-231 parental and 53BP1 KO cells under normal growth conditions. The cell cycle stages (G1, S/G2, and M) were determined by a combination of Cyclin A expression levels and DAPI staining patterns. Single z-plane images were acquired by sequential scanning using confocal microscopy. Scale bar is 10 µm. f Correlation between Cyclin A expression levels (expressed as mean fluorescence inten… Image collected and cropped by CiteAb from the following publication (), licensed under a CC BY license.



Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21208) in ICC/IF Immunofluorescence analysis of Donkey anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate was performed using A549 cells stained with alpha Tubulin (YL1/2) Rat Monoclonal Antibody (Product # MA1-80017). 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 2µg/mL Rat primary antibody for 3 hours at room temperature. Donkey anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate (Product # A-21208) was used at a concentration of 1µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panela:green). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Rhodamine Phalloidin (Product # R415, 1:300) (Panel c: red). 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.



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1192 References

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