Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 633
Excitation/Emission Max	631/650 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_2535719

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
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	4 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these goat anti-mouse IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against bovine IgG, goat IgG, rabbit IgG, rat IgG, human IgG, and human serum. 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.

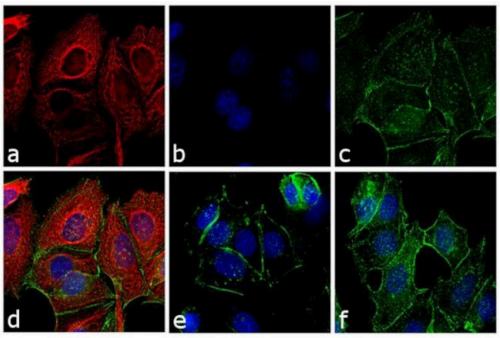
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 633 dye is a bright, far-

red-fluorescent dye with excitation ideally suited to the 633 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 633 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 633 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.

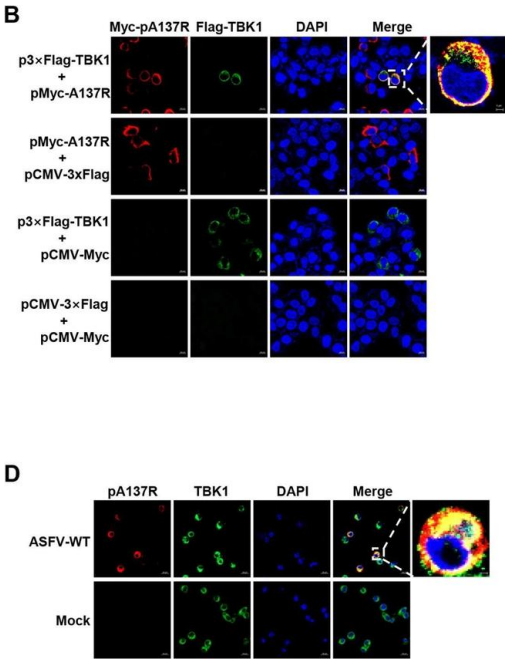
Product will be shipped at Room Temperature.

Product Images For Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633



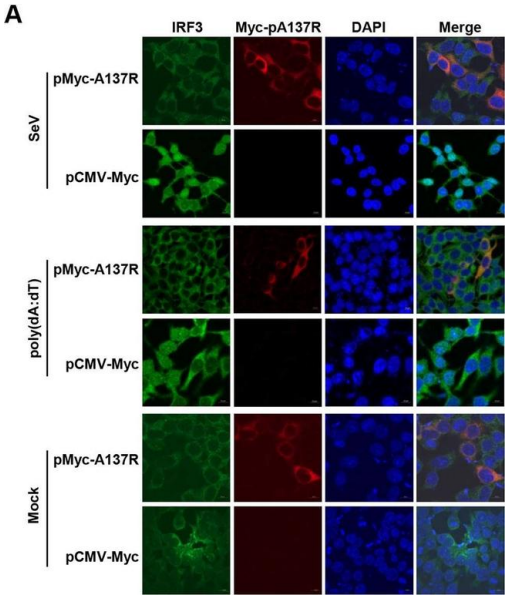
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21052) in ICC/IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 633 conjugate was performed using MCF-7 cells stained with Cytokeratin 19 (RCK108) Mouse Monoclonal Primary Antibody (Product # MA5-12613). 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 Mouse primary antibody (1:250 dilution) for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 633 conjugate (Product # A-21052) was used at a concentration of 4µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of Cytokeratin 19 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.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21052) in ICC/IF

The A137R protein (pA137R) interacts with TBK1. (A) HEK293T cells were transfected with p3×Flag-cGAS, -STING, -TBK1, or -IRF3 for 48 h and lysed with NP-40 buffer. The purified GST or GST-pA137R protein was used to pull down the key adaptors of the cGAS-STING pathway in the lysates and analyzed by Western blotting using mouse anti-GST or -Flag monoclonal antibody (MAb). (B) HEK293T cells were cotransfected with p3×Flag-TBK1 and pMyc-A137R for 24 h and then incubated with rabbit anti-Flag or mouse anti-Myc MAb and Alexa Fluor 488 (green)- or 633 (red)-conjugated secondary antibody, respectively. Cell nuclei (blue) were stained with 4',6-diamidino-2-phenylindole (DAPI). Bars, 10 µm. (C) Primary porcine alveolar macrophages (PAMs) were infected with the wild-type ASFV HLJ/2018 strain (ASFV-WT) at a multiplicity of infection (MOI) of 1 for a coimmunoprecipitation assay. The lysates were collected at 48 hours post-infection and incubated with protein G agarose, along with rabbit anti-pA137R polyclonal antibodies (PAbs) or irrelevant rabbit immunoglobulin G (IgG), and then the bound proteins were analyzed by Western blotting using in-house mouse anti-pA137R or rabbit anti-TBK1 PAbs. (D) PAMs were uninfected or infected with ASFV-WT for 24 h at an MOI of 1, and the localization of pA137R and TBK1 was visualized by laser confocal microscopy using in-house mouse anti-pA137R or rabbit anti-TBK1 PAbs and the indicated … Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35412346), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21052) in ICC/IF

The A137R protein (pA137R) blocks the nuclear translocation of interferon factor 3 (IRF3). HEK293T cells were transfected with pMyc-A137R or pCMV-Myc for 24 h and then stimulated with Sendai virus (SeV) at a multiplicity of infection of 1 or transfected with poly(dA-dT) (2 µg/mL) for 8 h. (A) The subcellular localization of IRF3 (green), pA137R (red), or cell nuclei (blue) was observed by laser confocal microscopy. Bars, 10 µm. (B) The transfected cells with IRF3 nuclear translocation were counted from 100 cells per condition from different fields in panel A. Error bars denote standard errors of the means. All the data were analyzed using Student's t test: *, $P < 0.05$; ns, not significant. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35412346), licensed under a CC BY license.

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523 References

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