Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555

Product Details	
Size	1 mg
Species Reactivity	Mouse
Published Species	Mouse
Host/Isotope	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor® 555
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_141780

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	1-10 µg/mL	-
Immunocytochemistry (ICC)	4 µg/mL	13 Publications
Immunofluorescence (IF)	4 µg/mL	1 Publication
Immunohistochemistry (IHC)	Assay Dependent	19 Publications
Immunohistochemistry (Frozen) (IHC (F))	-	3 Publications
Miscellaneous PubMed (Misc)	-	36 Publications
Western Blot (WB)	-	2 Publications

Product Specific Information

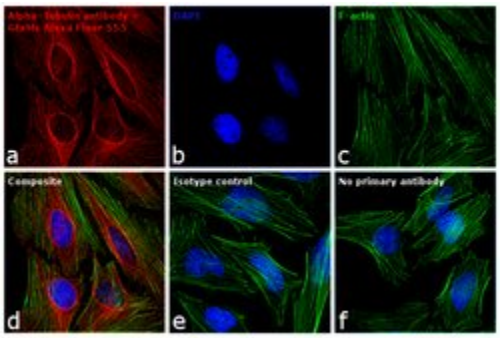
To minimize cross-reactivity, these goat anti-mouse IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against bovine IgG, goat IgG, rabbit IgG, rat IgG, human IgG, and human serum. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the 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 555 dye is a bright,

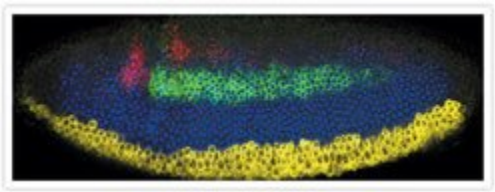
orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 555 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.

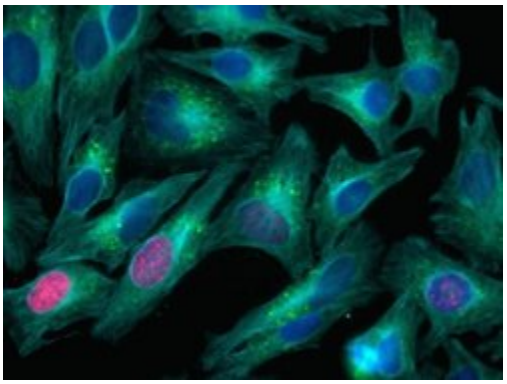
Product Images For Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424) in IF
Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 conjugate was performed using HeLa cells stained with alpha Tubulin (236-10501) Mouse Monoclonal Antibody (Product # A11126). 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 2 µg/mL primary antibody for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 (Product # A-21424) was used at a concentration of 4 µg/mL in phosphate buffered saline containing 0.2% BSA for 45 minutes at room temperature, for detection of alpha Tubulin 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-21424) in IF
Four-color fluorescence in situ hybridization on a Drosophila embryo. A late blastoderm stage (nuclear cycle 14) embryo was probed with four different RNA probes. Blue: sog labeled with DNP, followed by a rabbit anti-dinitrophenyl-KLH IgG antibody (Product # A-6430) detected with an Alexa Fluor® 647 chicken anti-rabbit IgG antibody (Product # A-21443). Green: ind labeled with biotin, followed by streptavidin HRP and Alexa Fluor® 350 tyramide (TSA Kit #27, Product # T-20937). Red: msh labeled with digoxigenin followed by sheep anti-digoxigenin antibody detected with an Alexa Fluor® 488 donkey anti-sheep IgG antibody (Product # A-11015). Yellow: sna labeled with FITC followed by mouse anti-FITC antibody detected with an Alexa Fluor® 555 goat anti-mouse IgG antibody (Product # A-21424). Image contributed by Dave Kosman and Ethan Bier, University of California, San Diego.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424) in IF
HeLa cells were treated with 30 µM chloroquine and cultured overnight. The following day, the cells were fed 10 µM EdU under regular growth conditions for one hour and then fixed and permeabilized. EdU was used to was visualize proliferating cells using The Click-iT® EdU Alexa Fluor® 488 Imaging kit (pink). Cells were counter stained with 0.5 µg/mL anti-LC3B with a goat anti rabbit Alexa Fluor® 647 secondary (Green), mouse anti alpha tubulin with a goat anti mouse Alexa Fluor® 555 secondary (Cyan) and 1 µg/mL Hoechst 33342 (Blue). Cells were imaged on a Molecular Devices ImageXpress High content imager.

View more figures on thermofisher.com

74 References

Immunohistochemistry (19)

BMC cancer

A novel spheroid-based co-culture model mimics loss of keratinocyte differentiation, melanoma cell invasion, and drug-induced selection of ABCB5-expressing cells.

"A-21424 was used in Immunohistochemistry-immunofluorescence to describe a novel, simple spheroid-based melanoma tri-culture model composed of fibroblasts, keratinocytes, and melanoma cells which mimicked features observed in early melanoma stages."

Authors: Klicks J,Maßlo C,Kluth A,Rudolf R,Hafner M

Species
Mouse
Not Applicable

Dilution
Not Cited
Not Cited

Year
2019

Cell reports

Endocardial Notch Signaling Promotes Cardiomyocyte Proliferation in the Regenerating Zebrafish Heart through Wnt Pathway Antagonism.

"A-21424 was used in Immunohistochemistry-immunofluorescence to nhibition of investigate if endocardial Notch signaling following ventricular amputation compromises cardiomyocyte proliferation and stimulates fibrosis."

Authors: Zhao L,Ben-Yair R,Burns CE,Burns CG

Species
Mouse
Not Applicable

Dilution
1:200
1:200

Year
2019

View more IHC references on thermofisher.com

Miscellaneous PubMed (36)

BMC cancer

A novel spheroid-based co-culture model mimics loss of keratinocyte differentiation, melanoma cell invasion, and drug-induced selection of ABCB5-expressing cells.

"A-21424 was used in Immunohistochemistry-immunofluorescence to describe a novel, simple spheroid-based melanoma tri-culture model composed of fibroblasts, keratinocytes, and melanoma cells which mimicked features observed in early melanoma stages."

Authors: Klicks J,Maßlo C,Kluth A,Rudolf R,Hafner M

Species
Mouse
Not Applicable

Dilution
Not Cited
Not Cited

Year
2019

Autophagy

TGFB1 is secreted through an unconventional pathway dependent on the autophagic machinery and cytoskeletal regulators.

"A-21424 was used in Immunocytochemistry-immunoflourescence to reveal that F-Box protein 32 (FBXO32) regulated ATR expression in pancreatic cancer PANC-1 and MIA PaCa-2 cells."

Authors: Nüchel J,Ghatak S,Zuk AV,Illerhaus A,Mörgelin M,Schönborn K,Blumbach K,Wickström SA,Krieg T,Sengle G, Plomann M,Eckes B

Species
Mouse
Not Applicable

Dilution
Not Cited
Not Cited

Year
2019

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More applications with references on thermofisher.com

ICC (13) **IHC (F) (3)** **WB (2)** **IF (1)**