Website: thermofisher.com
Customer Service (US): 1 800 955 6288 ext. 1
Technical Support (US): 1 800 955 6288 ext. 441
thermofisher.com/contactus

Performance guaranteed

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

Catalog Number A-21424

Product data sheet

Details	
Size	500µL
Host/Isotope	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Immunogen	Gamma Immunoglobins Heavy and Light chains
Target Class	IgG
Cross Adsorption	Against bovine IgG, goat IgG, rabbit IgG, rat IgG, human IgG and human serum
Antibody Form	Whole Antibody
Conjugate	Alexa Fluor® 555
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

Species Reactivity	
Tested species reactivity	Mouse
Published species reactivity	Not Applicable

Tested Applications	Dilution *
Flow Cytometry (Flow)	1-10 µg/mL
Immunocytochemistry (ICC)	1-10 µg/mL
Immunofluorescence (IF)	1-10 µg/mL

Published Applications	
Immunohistochemistry (Frozen) (IHC (F))	See 1 publications below
Miscellaneous PubMed (MISC)	See 13 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

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.

Background/Target Information

We offer an extensive line of Invitrogen™ secondary antibody conjugates with well-characterized specificity and labeled with a wide selection of premium fluorescent dyes, including Invitrogen™ Alexa Fluor™ fluorescent dyes. Fluorescent secondary antibody conjugates are useful in the detection, sorting, or

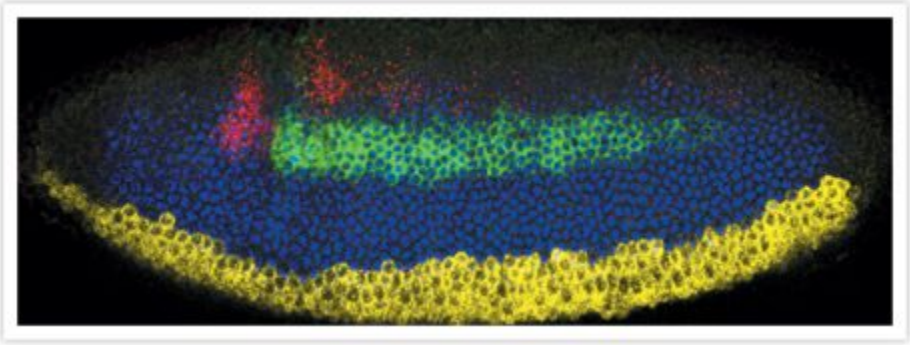
purification of its specified target and ideal for fluorescence microscopy and confocal laser scanning microscopy, flow cytometry, and fluorescent western detection. The breadth of fluorescent markers we offer allows our reagents to be tailored to almost any fluorescent detection system.Secondary antibodies may be provided in three formats: whole IgG, divalent F(ab')2 fragments, and monovalent Fab fragments. Because of the high degree of conservation in the structure of many immunoglobulin domains, most class-specific secondary antibodies must be affinity-purified and cross-adsorbed to achieve minimal cross-reaction with other immunoglobulins.Our secondary antibody conjugates are most commonly prepared by immunizing the host animal with a pooled population of immunoglobulins from the target species and can be further purified and modified (e.g., immunoaffinity chromatography, antibody fragmentation , label conjugation, etc.) to generate highly specific reagents. In the first round of purification, whole immunoglobulins binding to the immunizing antibody are recovered and mainly consist of the ~150-kDa IgG class. Further purification, for example, with Protein A or G, removes all unwanted immunoglobulin classes except the affinity-purified antibodies that react with the target-specific immunoglobulin heavy and/or light chains.

For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

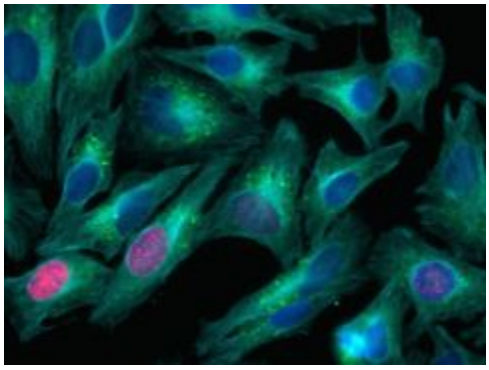
thermofisher.com/contactus

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



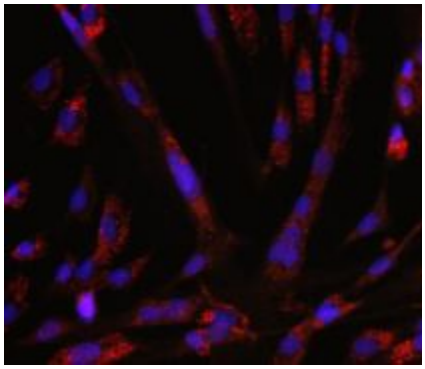
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424)

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 (Prod # A6430) detected with an Alexa Fluor® 647 chicken anti-rabbit IgG antibody (Prod # A21443). Green: ind labeled with biotin, followed by streptavidin HRP and Alexa Fluor® 350 tyramide (TSA Kit #27, Prod # T20937). Red: msh labeled with digoxigenin followed by sheep anti-digoxigenin antibody detected with an Alexa Fluor® 488 donkey anti-sheep IgG antibody (Prod # A11015). Yellow: sna labeled with fluorescein followed by mouse anti-fluorescein antibody detected with an Alexa Fluor® 555 goat anti-mouse IgG antibody (Prod # A21424). Image contributed by Dave Kosman and Ethan Bier, University of California, San Diego.



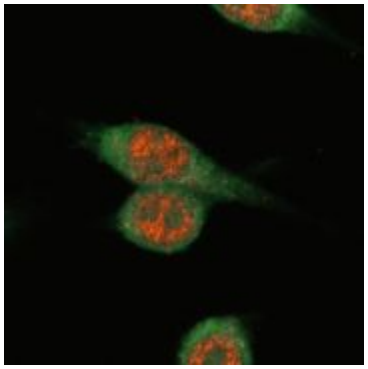
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424)

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.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424)

Gibco® Human skeletal muscle cells were treated with 30uM Chloroquine overnight. The following day the cells were fixed and permeabilized and labeled with 0.5ug/mL anti-LC3B with a goat anti mouse Alexa Fluor® 555 secondary (Red) Cells were counter stained with 1ug/mL Hoechst 33342 (Blue) Images were acquired with a BD Pathway 855 High content imager

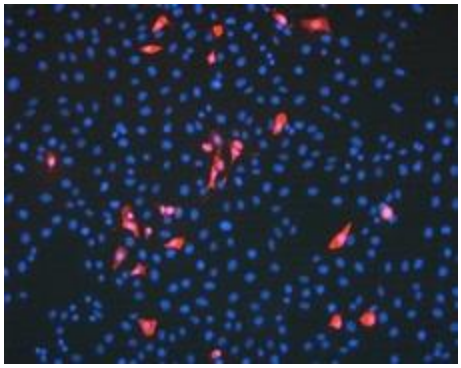


Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424)

Hemin induced lipid peroxidation and nrf-2 nuclear translocation in RAW macrophage cells. Detection of lipid peroxidation was done by click reaction with AlexaFluor® 488 azide using Click-iT® Lipid Peroxidation Imaging Kit - Alexa Fluor® 488 (Catalog No. C10446) and nrf-2 by using an antibody against nrf-2 and AlexaFluor® 555 secondary antibody.

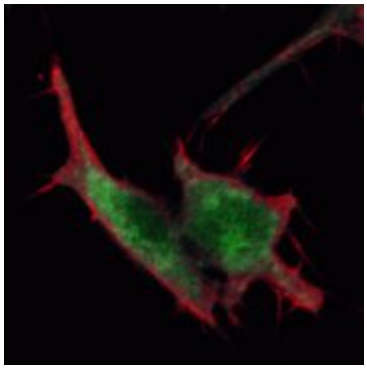
Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424)

Immunofluorescent analysis of Influenza A NP in infected Vero cells using an Influenza A NP monoclonal antibody (Product # HYB 156-01-02) at a dilution of 1:500 followed by detection using a secondary polyclonal goat anti-mouse Ig Fluor 555 conjugated antibody (Product # A21424) at a dilution of 1:200, and Vectashield mounting medium with DAPI.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424)

Hemin induced lipid peroxidation in bovine pulmonary artery endothelial cells. Detection of lipid peroxidation was by click reaction with AlexaFluor 488® azide and hydroxynonenal (HNE) was detected by using an antibody against HNE and AlexaFluor® 555 secondary antibody.

Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus

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

1 Immunohistochemistry (Frozen) References

Species / Dilution	Summary
	A-21424 was used in immunohistochemistry - frozen section to assess the effects of tissue-type plasminogen activator treatment after oral anticoagulation with rivaroxaban or apixaban compared with warfarin or placebo
Not Applicable / 1:500	Stroke; a journal of cerebral circulation (Aug 2014; 45: 2404) **"Rivaroxaban and apixaban reduce hemorrhagic transformation after thrombolysis by protection of neurovascular unit in rat."** Author(s):Kono S,Yamashita T,Deguchi K,Omote Y,Yunoki T,Sato K,Kurata T,Hishikawa N,Abe K PubMed Article URL:http://dx.doi.org/10.1161/STROKEAHA.114.005316

13 Miscellaneous PubMed References

Species / Dilution	Summary
Not Applicable / Not Cited	Cell cycle (Georgetown, Tex.) (Jan 2010; 9: 389) **"The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks."** Author(s):Nakamura AJ,Rao VA,Pommier Y,Bonner WM PubMed Article URL:http://dx.doi.org/10.4161/cc.9.2.10475
Not Applicable / Not Cited	Cancer research (Jul 2009; 69: 5860) **"High efficacy of a Listeria-based vaccine against metastatic breast cancer reveals a dual mode of action."** Author(s):Kim SH,Castro F,Paterson Y,Gravekamp C PubMed Article URL:http://dx.doi.org/10.1158/0008-5472.CAN-08-4855
Not Applicable / Not Cited	BMC biotechnology (May 2009; 9: null) **"Simultaneous detection of mRNA and protein stem cell markers in live cells."** Author(s):Rhee WJ,Bao G PubMed Article URL:http://dx.doi.org/10.1186/1472-6750-9-30
Not Applicable / Not Cited	The American journal of pathology (May 2009; 174: 1891) **"Protective role of endogenous gangliosides for lysosomal pathology in a cellular model of synucleinopathies."** Author(s):Wei J,Fujita M,Nakai M,Waragai M,Sekigawa A,Sugama S,Takenouchi T,Masliah E,Hashimoto M PubMed Article URL:http://dx.doi.org/10.2353/ajpath.2009.080680
Not Applicable / Not Cited	The Journal of cell biology (Apr 2009; 185: 305) **"Early endosomes and endosomal coatomer are required for autophagy."** Author(s):Razi M,Chan EY,Tooze SA PubMed Article URL:http://dx.doi.org/10.1083/jcb.200810098
Not Applicable / Not Cited	Journal of virology (Apr 2009; 83: 2872) **"Dual roles for an arginine-rich motif in specific genome recognition and localization of viral coat protein to RNA replication sites in flock house virus-infected cells."** Author(s):Venter PA,Marshall D,Schneemann A PubMed Article URL:http://dx.doi.org/10.1128/JVI.01780-08
Not Applicable / Not Cited	Nature methods (Mar 2009; 6: 207) **"High-efficiency labeling of sialylated glycoproteins on living cells."** Author(s):Zeng Y,Ramya TN,Dirksen A,Dawson PE,Paulson JC PubMed Article URL:http://dx.doi.org/10.1038/nmeth.1305
Not Applicable / Not Cited	American journal of physiology. Cell physiology (Dec 2008; 295: C1476) **"A fluorimetry-based ssYFP secretion assay to monitor vasopressin-induced exocytosis in LLC-PK1 cells expressing aquaporin-2."** Author(s):Nunes P,Hasler U,McKee M,Lu HA,Bouley R,Brown D PubMed Article URL:http://dx.doi.org/10.1152/ajpcell.00344.2008
Not Applicable / Not Cited	Journal of virology (Oct 2008; 82: 9477) **"The transmembrane domain of the severe acute respiratory syndrome coronavirus ORF7b protein is necessary and sufficient for its retention in the Golgi complex."** Author(s):Schaecher SR,Diamond MS,Pekosz A PubMed Article URL:http://dx.doi.org/10.1128/JVI.00784-08
Not Applicable / Not Cited	Science (New York, N.Y.) (Jun 2008; 320: 1332) **"Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy."** Author(s):Schermelleh L,Carlton PM,Haase S,Shao L,Winoto L,Kner P,Burke B,Cardoso MC,Agard DA,Gustafsson MG,Leonhardt H,Sedat JW PubMed Article URL:http://dx.doi.org/10.1126/science.1156947

Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus

Not Applicable / Not Cited	Proceedings of the National Academy of Sciences of the United States of America (Apr 2008; 105: 5774) **"Rare steroid receptor-negative basal-like tumorigenic cells in luminal subtype human breast cancer xenografts."** Author(s):Horwitz KB,Dye WW,Harrell JC,Kabos P,Sartorius CA PubMed Article URL:http://dx.doi.org/10.1073/pnas.0706216105
Not Applicable / Not Cited	Nature methods (Jan 2008; 5: 75) **"Resolution of de novo HIV production and trafficking in immature dendritic cells."** Author(s):Turville SG,Aravantinou M,Stössel H,Romani N,Robbiani M PubMed Article URL:http://dx.doi.org/10.1038/nmeth1137
Not Applicable / Not Cited	Journal of proteome research (Nov 2006; 5: 2956) **"Comparison of hydroxylated print additives on antibody microarray performance."** Author(s):Wu P,Grainger DW PubMed Article URL:http://dx.doi.org/10.1021/pr060217d

Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA