

Goat anti-Mouse IgM (Heavy chain) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

Product Details	
Size	500 µg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 488
Excitation/Emission Max	499/520 nm
Immunogen	Mouse Mu immunoglobulin
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_2535711

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	1 µg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these goat anti-mouse IgM whole secondary antibodies have been affinity purified and cross-adsorbed against human IgG1, IgG2, IgG3, IgG4, IgA, human serum, and purified human paraproteins prior to conjugation. 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.

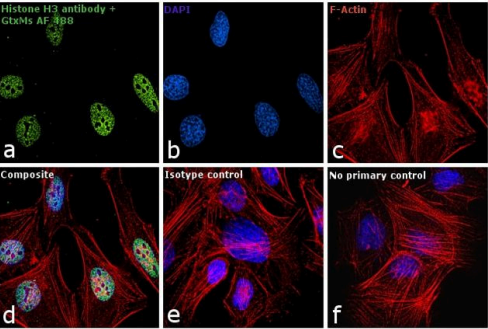
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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 IgM (Heavy chain) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

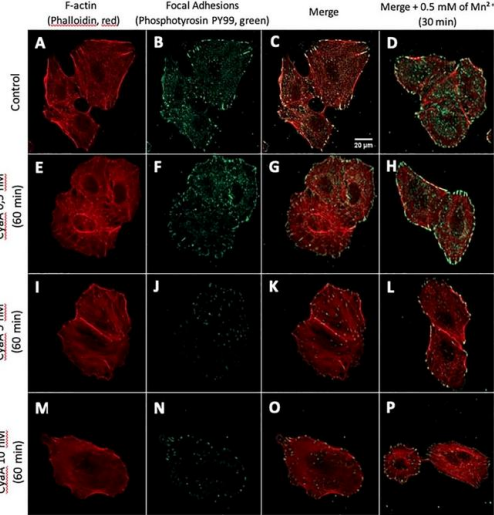
Mouse IgM (Heavy chain) Cross-Adsorbed Secondary Antibody (A-21042) in ICC/IF

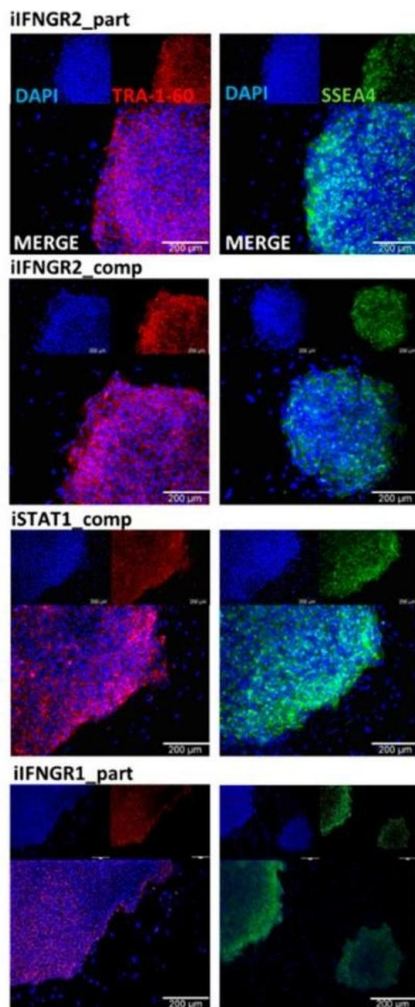
Immunofluorescence analysis of Goat anti-Mouse IgM Heavy Chain Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (A21042) was performed using HeLa cells stained with Histone H3 Mouse Monoclonal Antibody (AH01432). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 µg/mL of mouse primary antibody for 3 hours at room temperature. Goat anti-Mouse IgM Heavy Chain Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (A21042) was used at concentration of 1 µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of Histone (H3) in the nucleus (Panel a: green). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (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..



Mouse IgM (Heavy chain) Cross-Adsorbed Secondary Antibody (A-21042) in ICC/IF

Cell imaging of F-actin structure and focal adhesion points. Co-staining of F-actin (with phalloidin, in red) and of focal adhesions (with anti-phosphotyrosin PY99 antibody, in green) in fixed A549 cells (control conditions) after 1 hr of exposure to the indicated concentrations of CyaA toxin. (A, E, I, M) F-actin staining, (B, F, J, N) focal adhesion staining, (C, G, K, O) merge images. Right panels (D, H, L, P) show merged images of F-actin staining and focal adhesion staining for cells incubated with the indicated concentrations of CyaA for 60 min followed by a 30 min exposure to Mn2+ at a concentration of 0.5mM. (A-D) Control conditions (no CyaA), (E-H) cell exposure to 0.5nM of CyaA, (I-L) cell exposure to 5nM of CyaA, (M-P) cell exposure to 10nM of CyaA. Images were obtained by confocal microscopy with x63 magnification. S1 and S2 Figs further document the cell viability and intracellular cAMP accumulation in the A549 cells exposed to CyaA. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32392246>), licensed under a CC BY license.



B

Mouse IgM (Heavy chain) Cross-Adsorbed Secondary Antibody (A-21042) in ICC/IF

Further analysis of patient-iPSCs and thereof derived macrophages. (A) Sequence and electropherogram for the patient-specific mutation. Sequencing was performed on all patient lines for all patient mutations. The mutated nucleotide or insertion is highlighted in red. (B) Representative immunofluorescent staining for TRA-1-60 (left) and SSEA4 (right) in patient iPSCs co-stained with DAPI. Scale bar = 200 μm. (C) Representative western blot analysis of STAT1 expression in unstimulated samples and samples stimulated with a low (25 ng/mL) or high (100 ng/mL) dose of IFN. Tubulin was used as a loading control. (D) Genes that were significantly up- or downregulated ($p = 0.05$ in stimulated iIFNGR2_comp macrophages compared to the other groups) were analyzed via gProfiler for enriched WikiPathway groups. The top 10 groups are shown. (E) Flow cytometry analysis of healthy control and patient-specific iPSC-derived macrophages shows no difference between unstimulated (top row) and stimulated (bottom row) macrophages regarding their viability as evaluated by the FSC/SSC scatter. Quantification was performed for four independent experiments. ($n = 4$; mean \pm SD). Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32093117>), licensed under a CC BY license.

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456 References

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