# Chicken anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

| Product Details | |
|---|---|
| Size | 1 mg |
| Species Reactivity | Rabbit |
| Host/Isotype | Chicken / IgY |
| 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 | AB_2535859 |

| Applications | Tested Dilution | Publications |
|---|---|---|
| Western Blot (WB) | 1:2,000-1:2,500 | 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) | 1:1,000-1:2,000 | 0 Publication |
| Flow Cytometry (Flow) | - | 0 Publication |
| Miscellaneous PubMed (Misc) | - | 0 Publication |

### Product Specific Information

To minimize cross-reactivity, these chicken anti-rabbit IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human and mouse IgG 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 are may be the presence of endogenous immunoglobulins.

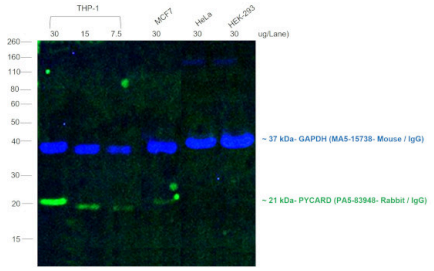
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright,

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.

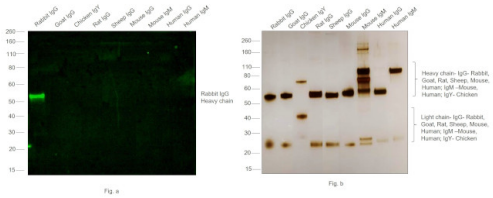
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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.

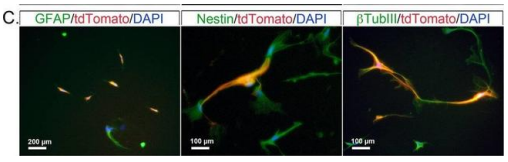
## Product Images For Chicken anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488




**Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21441) in WB**
Multiplexed fluorescent western blot was performed using Chicken anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (Product # A-21441). Membrane extracts of THP-1 (Lane 1, 2, 3), MCF7 (Lane 4), HeLa (Lane 5), and HEK-293 (Lane 6) were electrophoresed usingNuPAGE™ 12% Bis-Tris Protein Gel (Product # NP0341BOX). Resolved proteins were transferred onto aLow-Fluorescence PVDF Transfer Membranes, 0.2 m 7 x 8.4 cm (Product # 22860) byiBlot® 2 Dry BlottingSystem (Product # IB21001) and blocked using Blocker™ FL Fluorescent Blocking Buffer (10X) (Product # 37565). The blot was probed with PYCARD Polyclonal Antibody (Product # PA5-83948), and GAPDH Loading Control Monoclonal Antibody (GA1R) (Product # MA5-15738). Secondary antibodies (Product # A-21441, 1:2500), and (Product # A32789, 1: 10,000) were used for detection of PYCARD, and GAPDH respectively. Fluorescent detection was performed usingiBright™FL1500 (Product # A44115). The anti-rabbit secondary antibody (Product # A-21441) specifically detects the rabbit primary antibody.



**Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21441)**
Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rabbit IgG. Band at ~55 kDa corresponding to Rabbit IgG Heavy Chain was observed in Rabbit IgG but not in other species using Chicken anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (Product # A-21441) in Western Blot. {RE}




**Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21441) in ICC/IF**
Mouse DFAT cells derived from combined BAT and WAT from Adipoq-cre/ERT; tdTomato mice after Tamoxifen induction. (A) TdTomato subcutaneous adipose tissue and mixed DFAT cells from Adipoq-cre/ERT2;tdTomato mice. (B) Bright field images of tdTomato DFAT cells after 15 days of culture in neural induction medium. (C) Co-localization of tdTomato (red) and the neurogenic markers glial fibrillary acidic protein (GFAP, green), Nestin (green), and tubulin III (TubIII, green) in DFAT cells after 15 days of culture in neural induction medium as visualized by immunofluorescence. (D) Co-localization of tdTomato and S100 Calcium Binding Protein B (S100, green) in aggregates of tdTomato-positive DFAT cells as visualized by immunofluorescence. (E) Staining for TubIII and GFAP in sections from DFAT cell clusters formed in 3D-Matrigel. DAPI (blue) was used to visualize nuclei. Representative images from 3-5 experiments. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35379860), licensed under a CC BY license.

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## 444 References

Monocarboxylate transporter 4 deficiency enhances high-intensity interval training-induced metabolic adaptations in skeletal muscle. J Physiol (2024)

Tenascin C in pancreatic cancer-associated fibroblasts enhances epithelial mesenchymal transition and is associated with resistance to immune checkpoint inhibitor. Am J Cancer Res (2023)

Generation of chimeric antigen receptor macrophages from human pluripotent stem cells to target glioblastoma. Immunooncol Technol (2023)

Loss of Cadherin-11 in pancreatic ductal adenocarcinoma alters tumor-immune microenvironment. Front Oncol (2023)

Depletion of slow-cycling PDGFR+ADAM12+ mesenchymal cells promotes antitumor immunity by restricting macrophage efferocytosis. Nat Immunol (2023)