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Datasheet for ABIN101988

# Goat anti-Rabbit IgG (Heavy & Light Chain) Antibody (FITC) - Preadsorbed

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## Overview

| | |
|---|---|
| Quantity: | 2 mg |
| Target: | IgG |
| Binding Specificity: | Heavy & Light Chain |
| Reactivity: | Rabbit |
| Host: | Goat |
| Clonality: | Polyclonal |
| Conjugate: | FITC |
| Application: | Flow Cytometry (FACS), FLISA, Fluorescence Microscopy (FM) |

## Product Details

| | |
|---|---|
| Immunogen: | Immunogen: Rabbit IgG whole molecule |
| Isotype: | IgG |
| Specificity: | IgG (H&L) |
| Cross-Reactivity: | Rabbit |
| Characteristics: | Concentration Definition: by UV absorbance at 280 nm |
| Purification: | Preadsorption: Solid phase absorption |
| Labeling Ratio: | 3.1 |

## Target Details

| | |
|---|---|
| Target: | IgG |

 

## Target Details

| | |
|---|---|
| Abstract: | IgG Products |
| Target Type: | Antibody |
| Background: | Synonyms: Goat anti-Rabbit IgG Antibody fluorescein Conjugation, Goat anti-Rabbit IgG FITC Conjugated Antibody<br>Background: Anti-Rabbit IgG Antibody Fluorescein generated in goat detects rabbit IgG. Secreted as part of the adaptive immune response by plasma B cells, immunoglobulin G constitutes 75 % of serum immunoglobulins. Immunoglobulin G binds to viruses, bacteria, as well as fungi and facilitates their destruction or neutralization via agglutination (and thereby immobilizing them), activation of the compliment cascade, and opsinization for phagocytosis. The whole IgG molecule possesses both the F(c) region, recognized by high-affinity Fc receptor proteins, as well as the F(ab) region possessing the epitope-recognition site. Both heavy and light chains of the antibody molecule are present. Secondary Antibodies are available in a variety of formats and conjugate types. When choosing a secondary antibody product, consideration must be given to species and immunoglobulin specificity, conjugate type, fragment and chain specificity, level of cross-reactivity, and host-species source and fragment composition. This Anti-Rabbit IgG (H&L) is conjugated to Fluorescein. |

## Application Details

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|---|---|
| Application Notes: | Application Note: This product is designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. This product is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms.<br>FLISA Dilution: 1:10,000 - 1:50,000<br>Flow Cytometry Dilution: 1:500 - 1:2,500<br>IF Microscopy Dilution: 1:1,000 - 1:5,000 |
| Comment: | Excitation/Emission wavelength: 494 nm/514 nm |
| Restrictions: | For Research Use only |

## Handling

| | |
|---|---|
| Format: | Lyophilized |
| Reconstitution: | Reconstitution Volume: 1.0 mL<br>Reconstitution Buffer: Restore with deionized water (or equivalent) |
| Concentration: | 2.0 mg/mL |

## Handling

| | |
|---|---|
| Buffer: | Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2<br>Stabilizer: 10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free<br>Preservative: 0.01 % (w/v) Sodium Azide |
| Preservative: | Sodium azide |
| Precaution of Use: | This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only. |
| Handling Advice: | Product is photosensitive and should be protected from light. |
| Storage: | RT,4 °C,-20 °C |
| Expiry Date: | 12 months |

## Publications

Product cited in:

Luo, Xiang, Lu, Tan, Li, Huang: "Association between dietary selenium intake and the prevalence of osteoporosis and its role in the treatment of glucocorticoid-induced osteoporosis." in: **Journal of orthopaedic surgery and research**, Vol. 18, Issue 1, pp. 867, (2023) (PubMed).

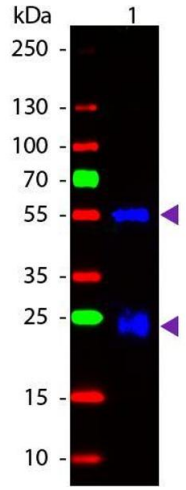
Fu, Pang, He, Zhang, Fan, Zhao, Yang: "Dexmedetomidine Confers Protection Against Neuronal Oxygen Glucose Deprivation-Reperfusion by Regulating SIRT3 Mediated Autophagy." in: **Neurochemical research**, Vol. 47, Issue 11, pp. 3490-3505, (2022) (PubMed).

Cong, Gong, Yang, Xia, Zhang: "MiR-200c/FUT4 axis prevents the proliferation of colon cancer cells by downregulating the Wnt/β-catenin pathway." in: **BMC cancer**, Vol. 21, Issue 1, pp. 2, ( 2021) (PubMed).

Li, Wang, Yang, Chu: "miR-142-3p targets AC9 to regulate sciatic nerve injury-induced neuropathic pain by regulating the cAMP/AMPK signalling pathway." in: **International journal of molecular medicine**, Vol. 47, Issue 2, pp. 561-572, (2021) (PubMed).

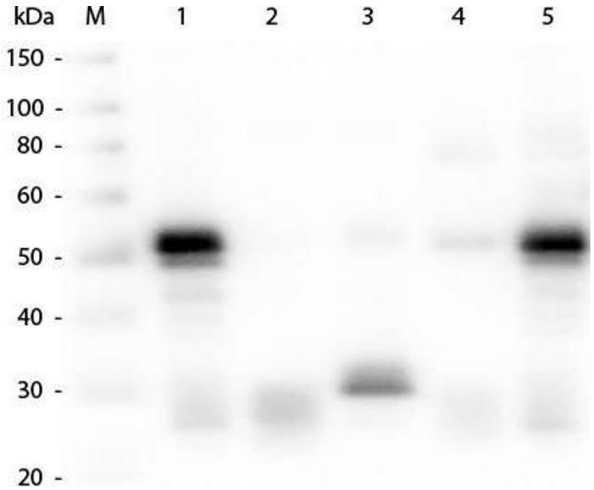
Faruk, Ibrahim, Aminu, Adamu, Abdullahi, Suleiman, Rafindadi, Mohammed, Iliyasu, Idoko, Saidu, Randawa, Musa, Ntekim, Shah, Abubakar, Adoke, Manko, Awasum: "Prognostic significance of BIRC7/Livin, Bcl-2, p53, Annexin V, PD-L1, DARC, MSH2 and PMS2 in colorectal cancer treated with FOLFOX chemotherapy with or without aspirin." in: **PLoS ONE**, Vol. 16, Issue 1, pp. e0245581, (2021) (PubMed).

There are more publications referencing this product on: Product page



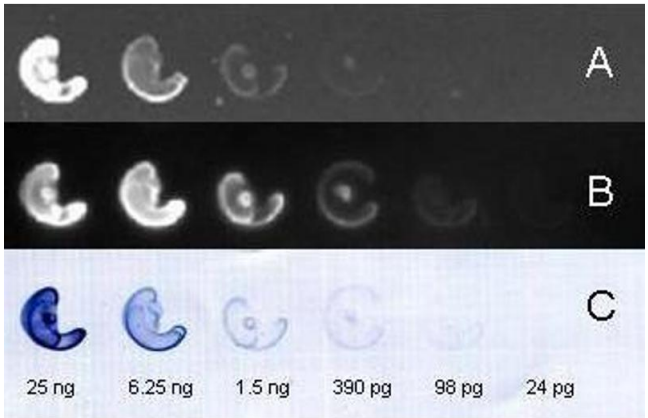

## Western Blotting

**Image 1.** Western blot of Fluorescein conjugated Goat Anti-Rabbit IgG secondary antibody. Lane 1: Rabbit IgG. Lane 2: None. Load: 50 ng per lane. Primary antibody: None. Secondary antibody: Fluorescein goat secondary antibody at 1:1,000 for 60 min at RT. Blocking: ABIN925618 for 30 min at RT. Predicted/Observed size: 25 & 55 kDa, 25 & 55 kDa for Rabbit IgG. Other band(s): None.




## Western Blotting

**Image 2.** Western Blot of Anti-Rabbit IgG (H&L) (GOAT) Antibody . Lane M: 3 µl Molecular Ladder. Lane 1: Rabbit IgG whole molecule . Lane 2: Rabbit IgG F(ab) Fragment . Lane 3: Rabbit IgG F(c) Fragment . Lane 4: Rabbit IgM Whole Molecule . Lane 5: Normal Rabbit Serum . All samples were reduced. Load: 50 ng per lane. Block: ABIN925618 for 30 min at RT. Primary Antibody: Anti-Rabbit IgG (H&L) (GOAT) Antibody 1:1,000 for 60 min at RT. Secondary antibody: Anti-Goat IgG (DONKEY) Peroxidase Conjugated Antibody 1:40,000 in ABIN925618 for 30 min at RT. Predicted/Obsevered Size: 25 and 50 kDa for Rabbit IgG and Serum, 25 kDa for F(c) and F(ab), 70 and 23 kDa for IgM. Rabbit F(c) migrates slightly higher.




## Dot Blot

**Image 3.** FITC (fluorescein) and HRP (horse radish peroxidase) conjugated secondary antibody was used to detect nanogram – picogram levels of rabbit IgG by dot blot on nitrocellulose membrane. 4 ul each of serial 1 in 4 dilutions of rabbit IgG were dotted on nitrocellulose and allowed to dry. Membrane was blcoked in 3% BSA for 10 minutes dried for later use and rewetted with ABIN925618. Blot was incubated in fluorescein conjugated goat anti rabbit lot 25176 1:10,000 and HRP conjugated goat anti

Rabbit (611-1302 lot 25406 1:10,000, dried and: A. Blot was imaged on the BioRad VersaDoc with filter settings appropriate for Fluorescein/DyLight 488 B. Blot was rewetted with TBS, incubated with FEMTOMAX chemiluminescent substrate for 1-3 minutes and imaged for 60sec on the BioRad VersaDoc Imaging System C. Blot was rinsed with TBS and DIH2O, incubated for 5 minutes with TMB Substrate for Western Blot MaxTag (1 ml of TMBM-102 + ~9 ml of TMBM-101), dried overnight and imaged using a conventional flatbed scanner

# Validation report #100071 for Immunofluorescence (IF)


**Successfully validated (Immunofluorescence (IF))**

by Okeanos Research Laboratory, Department of Biological Sciences, Clemson University

Report Number: 100071

Date: Sep 14 2016

| | |
|---|---|
| Target: | Rabbit IgG (Heavy & Light Chain) |
| Lot Number: | 611-1202 |
| Method validated: | Immunofluorescence (IF) |
| Positive Control: | Lab stock CBD-SNAP antibody |
| Negative Control: | No SNAP-tag antibody |
| Notes: | We validate the specificity of the secondary goat anti-rabbit IgG (heavy & light chain) antibody (FITC) ABIN101988 for rabbit IgG antibody. |
| Primary Antibody: | ABIN1573927 |
| Secondary Antibody: | ABIN101988 |

Protocol:

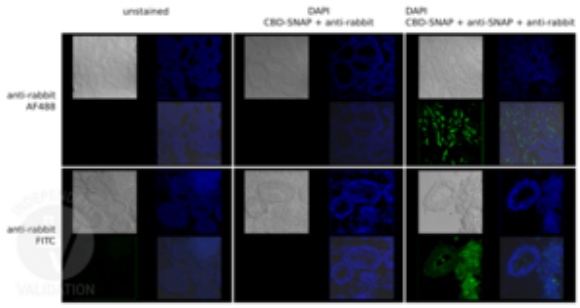
- Oyster visceral mass tissue is dissected and fixed in 4% paraformaldehyde in seawater overnight.
- Serial dehydration process using an automated ASP300S Enclosed Tissue Processor (Leica Biosystems) as follows:
  - 70% ethanol for 45min
  - 90% ethanol for 45min
  - 90% ethanol for 45min
  - 100% ethanol twice for 45min
  - xylene twice for 45min
  - paraffin wax at 58°C 3 times for 30 min
- Tissue is mounted in a paraffin block and hardened overnight before.
- 8µm tissue sections are retrieved from the block and collected on circular glass cover slips.
- Heat cover slips at 60°C for 1h.
- Deparaffination and rehydration:
  - Xylene twice for 15min
  - 100% ethanol twice for 10min
  - 95% ethanol for 10 min
  - 85% ethanol for 10 min
  - 70% ethanol for 10 min
  - 50% ethanol for 10 min
  - 30% ethanol for 10 min

- distilled water for 10 min
- PBS for 10 min

- Wash tissue sections with PBS with 0.05% triton X twice for 30min.
- Permeabilize in PBS with 0.05% triton X overnight.
- Treatment of the tissue sections with 1mg/mL sodium borohydride in PBS three times for 5min to reduce autofluorescence.
- Wash sections in PBS 3 times for 15 min for at RT.
- Block sections in PBST with 1% BSA for 2 hours at RT.
- Incubate sections with CBD-SNAP antibody (lab stock) diluted 1:200 in PBST with 1% BSA overnight at 4°C to detect the location of chitin.
- Wash sections in PBS 3 times for 15min with PBS at RT.
- Additionally, incubate the CBD-SNAP and SNAP-tag double-stained sections with rabbit anti-SNAP antibody (antibodies-online, ABIN1573927, lot 13D000621) diluted 1:200 in PBST with 1% BSA overnight at 4°C.
- Wash sections in PBS 3 times for 15min with PBS at RT.
- Incubate sections with the secondary goat anti-rabbit IgG (heavy & light chain) antibody (FITC) (antibodies-online, ABIN101988, lot 611-1202) diluted 1:400 in PBST with 1% BSA for 2h at °C.
- Wash sections in PBS three times for 15min at RT.
- Counterstain with 0.1µg/mL DAPI in PBS for 15min at RT.
- Wash sections in PBS three times for 15min at RT.
- Mount sections on a microscopic slide using 50% glycerol in PBS.
- Seal cover slips with nail polish.
- Confocal imaging on Leica SPE.
- Visualization of the data performed on LAS 3D software.

Experimental Notes:

To validate the specificity of the anti-rabbit FITC secondary antibody ABIN101988, 8µm paraffin sections of oyster visceral mass were observed in this study. We compared the fluorescence signals with immunofluorescence study. The negative control specimen was always compared with the test specimen or the positive control specimen on the same day, using the same laser power, gain, offset, accumulation/averaging settings on the Leica SPE confocal microscope. Visualization of the data was performed on LAS 3D software, with the same visualization setting to compare signal brightness. We found that the samples treated with anti-rabbit FITC secondary antibody ABIN101988 had similar fluorescence signals as the positive control Anti Rabbit Alexa 488. Excitation at the same laser wavelength and power did not generate fluorescence in the negative control section, when anti-rabbit FITC secondary antibody was applied in the absence of rabbit produced anti-SNAP antibody.




**Validation image no. 1 for Goat anti-Rabbit IgG (Heavy & Light Chain) antibody (FITC) - Preadsorbed (ABIN101988)**

Immunofluorescence images of oyster visceral mass tissue, with the specificity of Anti-Rabbit Alexa 488 (top row) and Anti-Rabbit FITC (bottom row) compared. Unstained samples (left column) were compared to test samples (right column) to visualize degree of auto-fluorescence. The absence of anti-SNAP served as the negative control (middle column), it has led to the absence of fluorescence at the same imaging and visualization setting for the green channels, implying the goat anti-rabbit IgG (Heavy & Light Chain) antibody FITC conjugate ABIN101988 is specific to Rabbit IgG antigen.