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Datasheet for 600-901-215

# GFP Antibody

## Overview

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| **Description:** | Anti-GFP (CHICKEN) Antibody - 600-901-215 |
| **Item No.:** | 600-901-215 |
| **Size:** | 100 µg |
| **Applications:** | Dot Blot, ELISA, WB, EM, IF, IHC, IP, Multiplex, Purification |
| **Reactivity:** | GFP, eGFP, rGFP |
| **Host Species:** | Chicken |

## Product Details

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| **Background:** | Anti-GFP antibody is designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. GFP antibody is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms. Chicken IgY lacks the classic "Fc" domain and does not bind to mammalian IgG Fc receptors. Thus resulting in lower backgrounds for western blotting, ELISA and Immunohistochemistry. |
| **Synonyms:** | chicken anti-GFP antibody, Green Fluorescent Protein, GFP antibody, Green Fluorescent Protein antibody, EGFP, enhanced Green Fluorescent Protein, Aequorea victoria, Jellyfish |
| **Host Species:** | Chicken |
| **Clonality:** | Polyclonal |
| **Format:** | IgY |

## Target Details

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| **Reactivity:** | GFP, eGFP, rGFP |
| **Immunogen Type:** | Recombinant Protein |
| **Immunogen:** | The immunogen is a Green Fluorescent Protein (GFP) fusion protein corresponding to the full length amino acid sequence (246aa) derived from the jellyfish Aequorea victoria. |

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| **Purity/Specificity:** | Anti-GFP IgY was prepared from egg yolks by immunoaffinity chromatography using Green Fluorescent Protein (Aequorea victoria) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Chicken Serum and purified and partially purified Green Fluorescent Protein (Aequorea victoria). No reaction was observed against Human, Mouse or Rat serum proteins. |
| **Relevant Links:** | • 600-901-215 SDS |

## Application Details

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| **Tested Applications:** | Dot Blot, ELISA, WB |
| **Suggested Applications:** | EM, IF, IHC, IP, Multiplex, Purification (Based on references) |
| **Application Note:** | Polyclonal anti-GFP is designed to detect GFP and its variants. Anti-GFP Antibody has been tested by ELISA, dot blot, and western and is suitable in immunofluorescence. This antibody can be used to detect GFP by ELISA (sandwich or capture) for the direct binding of antigen and recognizes wild type, recombinant and enhanced forms of GFP. Biotin conjugated polyclonal anti-GFP used in a sandwich ELISA is well suited to titrate GFP in solution using this antibody in combination with Rockland's monoclonal anti-GFP (600-301-215) using either form of the antibody as the capture or detection antibodies. However, use the monoclonal form only for the detection of wild type or recombinant GFP as this form does not sufficiently detect 'enhanced' GFP. The detection antibody is typically conjugated to biotin and subsequently reacted with streptavidin conjugated HRP (code # S000-03). Fluorochrome conjugated polyclonal anti-GFP can be used to detect GFP by immunofluorescence microscopy in prokaryotic (E.coli) and eukaryotic (CHO cells) expression systems and can detect GFP containing inserts. Significant amplification of signal is achieved using fluorochrome conjugated polyclonal anti-GFP relative to the fluorescence of GFP alone. For immunoblotting use either alkaline phosphatase or peroxidase conjugated polyclonal anti-GFP to detect GFP or GFP containing proteins on western blots. |
| **Assay Dilutions:** | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below. |
| **ELISA:** | 1:10,000 - 1:80,000 |
| **IF:** | User Optimized |
| **IHC:** | 1:200 - 1:1,000 |
| **WB:** | 1:1,000 - 1:5,000 |

## Formulation

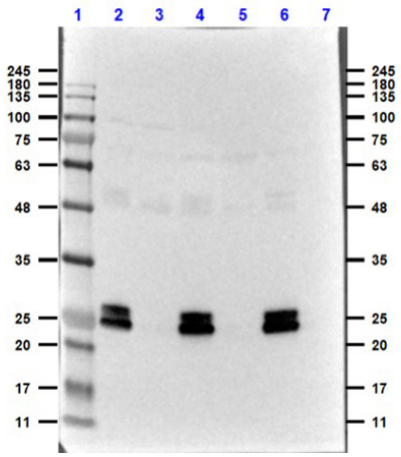
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| **Physical State:** | Liquid (sterile filtered) |
| **Concentration:** | 1.18 mg/ml by UV absorbance at 280 nm |

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| **Buffer:** | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 |
| **Preservative:** | 0.01% (w/v) Sodium Azide |
| **Stabilizer:** | None |

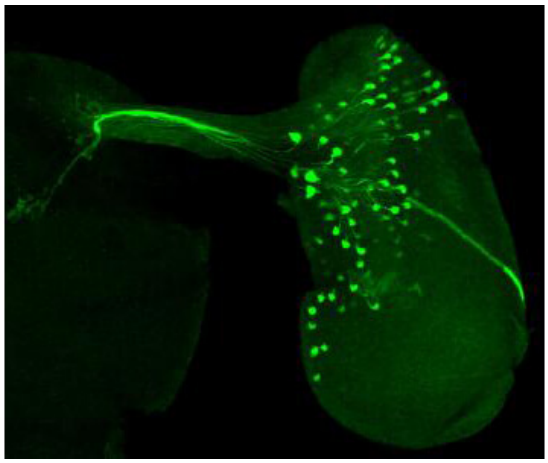
## Shipping & Handling

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| **Shipping Condition:** | Dry Ice |
| **Storage Condition:** | Store GFP Antibody at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. |
| **Expiration:** | Expiration date is one (1) year from date of receipt. |

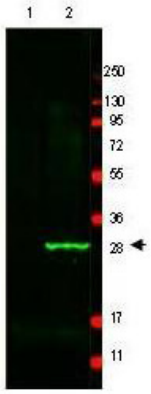
## Images



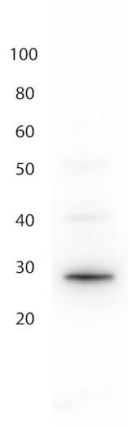

**Western Blot**
Western Blot of Chicken anti-GFP Antibody with Serums.
Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500).
Lane 2: GFP/Human Serum (p/n 000-001-215/D314-05) [0.01/0.02µL] [+].
Lane 3: Human Serum (p/n D314-05) [0.02µL] [-].
Lane 4: GFP/Mouse Serum (p/n 000-001-215/D308-05) [0.01/0.02µL] [+].
Lane 5: Mouse Serum (p/n D308-05) [0.02µL] [-].
Lane 6: GFP/Rat Serum (p/n 000-001-215/D310-05) [0.01/0.02µL] [+].
Lane 7: Rat Serum (p/n D310-05) [0.02µL] [-].
Primary antibody: Anti-GFP antibody at 1.0ug/mL overnight at 2-8°C.
Secondary antibody: Donkey Anti-Chicken IgG HRP secondary antibody (p/n 603-103-126) at 1:40,000 for 30 min at RT.
Blocking Buffer: BlockOut Buffer (p/n MB-073) for 1hr at RT.
Predicted/Observed size: ~27kDa for GFP.



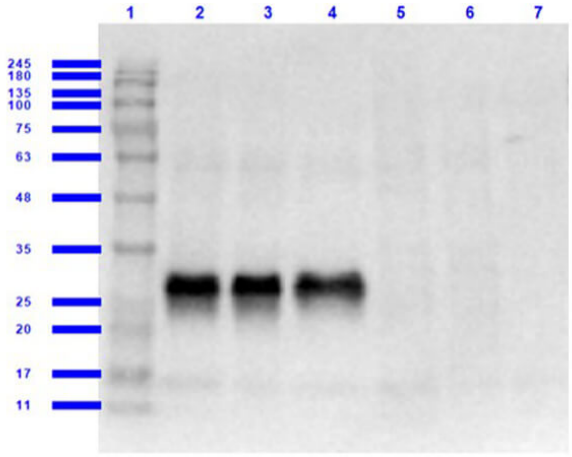
**Immunofluorescence Microscopy**
Immunofluorescence Microscopy of chicken anti-GFP antibody. Tissue: KruppleGAL4 driver line in Drosophila eye disc. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: anti-GFP antibody diluted 1:500 for 2 hr at RT. Secondary antibody: Alexa™488 conjugated anti-Chicken IgG at 1:300 for 1 hr at RT. Blocking: 5% NGS in PBS with 0.1% Triton X-100 for 15 min. Staining: recombinant tau-myc-GFP protein as green fluorescent signal.




**Western Blot**
Western Blot of anti-GFP antibody. Lane 1: mouse spleen lysate (p/n W10-000-T029). Lane 2: mouse spleen lysate spiked with 50 ng of wt GFP (p/n W10-000-T029/000-001-215). Load: 20 µg per lane. Primary antibody: GFP antibody at 2 µg/ml for 2 hr at room temperature. Secondary antibody: IRDye™800 Conjugated Affinity Purified anti-Chicken IgG [H&L] [Goat] MX10 (p/n 603-132-126) at 1:20,000 for 45 min at RT. Block: 5% BSA in PBS 2 hr at room temperature. Predicted/Observed size: 27 kDa for GFP epitope tag. Other band(s): none.




**Western Blot**
Western Blot of Anti-GFP (CHICKEN) antibody. Lane 1: MW. Lane 2: GFP (p/n 000-001-215). Load: 0.05 µg. Primary antibody: Anti-GFP (CHICKEN) antibody (p/n 600-901-215) was used at 1:1000 overnight at 4°C. Secondary antibody: Anti-Chicken IgG (GOAT) peroxidase conjugated antibody (p/n 603-103-126) secondary antibody was used at 1:40,000 in Blocking Buffer for Fluorescent Western Blotting (p/n MB-070). Block: 1% BSA-TTBS (p/n MB-013, diluted to 1X) 30 min at 20°C. Predicted/Observed size: 27 kDa for GFP. Other band(s): none.




**Western Blot**
Western Blot Results of Chicken Anti-GFP Antibody. Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 2: GFP (p/n 000-001-215) / HeLa WCL (p/n W09-000-364) [0.05µg/10µg]. Lane 3: GFP (p/n 000-001-215) / NIH3T3 WCL (p/n W10-000-371) [0.05µg/10µg]. Lane 4: GFP (p/n 000-001-215) / PC-12 WCL (p/n W120-001-GL9) [0.05µg/10µg]. Lane 5: HeLa WCL (p/n W09-000-364) [10µg]. Lane 6: NIH3T3 WCL (p/n W10-000-371) [10µg]. Lane 7: PC-12 WCL (p/n W120-001-GL9) [10µg]. Primary Antibody: Anti-GFP at 1.0µg/mL overnight at 2-8°C. Secondary Antibody: Goat Anti-Chicken IgG HRP (p/n 603-103-126) at 1:40,000 for 30 mins at RT. Block: MB-070 Buffer for 1hr at RT. Predicted MW: 27kda. Exposure: 2 seconds.

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