

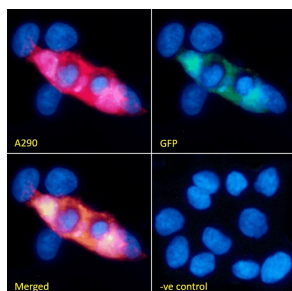
Anti-GFP Antibody (A290)

Specifications:

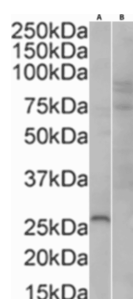
Name:	Anti-GFP Antibody
Description:	Rabbit polyclonal antibody to GFP.
Specificity:	This antibody is reactive against all variants of Aequorea victoria GFP such as S65T-GFP, RS-GFP, YFP, CFP, RFP, and EGFP.
Applications:	WB, ELISA, ICC/IF, Flow Cytometry, IHC-Fr, IHC-P, IP, Electron Microscopy
Positive Control:	Recombinant Aequorea victoria GFP protein, any other purified recombinant GFP protein, and any cell line confirmed to overexpress GFP.
Recommended Dilutions:	WB: 1:1,000-1:2,500, ICC: 1:200-1:1,000, Flow Cytometry: 1:100, IHC-P: 1:500-1:1,000, IHC-Fr: 1:500-1:2,000, Electron Microscopy: 1:1,000-1:4,000
Reactivity:	Species independent
Immunogen:	Recombinant full-length protein corresponding to Green Fluorescent Protein (GFP) from Aequorea victoria.
Sequence:	MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMLLEFVTAAGITHGMDELYK
Host:	Rabbit
Clonality:	Polyclonal
Isotype:	IgG
Conjugate:	Unconjugated
Purity:	Whole antiserum.
Product Form:	Liquid
Formulation:	Supplied as an aliquot of serum with 0.05% Sodium Azide and 1.25% Sodium Chloride.
Storage:	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid freeze / thaw cycles.
General Notes:	The total IgG concentration has been determined to be 5 mg/ml, however, as this antibody is provided as whole antiserum, the specific IgG concentration is unknown.
Disclaimer:	This product is for research use only. It is not intended for diagnostic or therapeutic use.

Anti-GFP Antibody (A290)

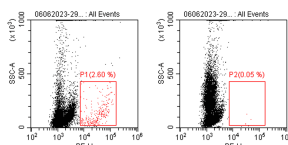
Images:



Immunofluorescence analysis of 4% paraformaldehyde fixed HEK293 cells transfected with GFP expressing plasmid, permeabilized with 0.15% Triton, using Anti-GFP Antibody (A290) at a 1:200 dilution (1 hour incubation), followed by Goat Anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (Alexa Fluor 568) (red) at a 1:1,000 dilution. The nuclear stain is DAPI (blue).



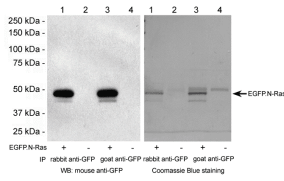
Western blot analysis of HEK293 cell lysates (30µg protein in RIPA buffer) using Anti-GFP Antibody (A290) at a 1:5,000 dilution (1 hour incubation). The lanes contain: (A) HEK293 cells transfected with GFP expressing plasmid, (B) non-transfected HEK293 cells. Detected by chemiluminescence.



Flow cytometry analysis of 2% paraformaldehyde fixed HEK293 cells transfected with GFP expressing plasmid (Left Panel), permeabilized with 0.5% Triton, using Anti-GFP Antibody (A290) at a 1:100 dilution (1 hour incubation), followed by Goat Anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (Alexa Fluor 568) (red) at a 1:200 dilution. Negative control: Non-transfected HEK293 cells (Right Panel).

Anti-GFP Antibody (A290)

Images continued:



Lane 1 & 3: IP from COS-7 cells transfected with EGFP.N-Ras (48 kDa). Lane 2 & 4: IP from untransfected COS 7 cells. Lane 1 & 2: IP using 15µl of Anti-GFP Antibody (A290) conjugated to sepharose beads (0.5 mg IgG per ml of beads). Lane 3 & 4: IP using 15 µl of an unpublished goat polyclonal antibody to GFP conjugated to sepharose beads (1 mg IgG per ml of beads). Immunoprecipitation: COS-7 cell lysates containing 100µg of total protein in 200µl of 0.1% SDS-RIPA buffer with addition of complete protease inhibitor were used for each immunoprecipitation. Cell lysates were incubated with Anti-GFP sepharose beads for 2 hours at 4°C with rocking. Beads were washed. Proteins were eluted with 1% SDS 50 mM HEPES (pH 7.4) at 80°C (15 min). Half of the IP sample was loaded on each lane of 10 % SDS PAGE gel and gel was processed for Western blotting/ECL. Western Blot: Primary Antibody: Anti-GFP antibody [LGB-1] (ab291) at 0.2µg/ml in 5% non-fat milk/TBS-T (1 hour incubation at room temperature). Secondary Antibody: Sheep Anti-Mouse IgG Antibody (HRP) at a 1:20,000 dilution in 5% non-fat milk/TBS-T (1 hour incubation at room temperature). Development with ECL plus (GE Healthcare). Exposure time: 5 seconds.