GFP Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF4240

DESCRIPTION

Specificity	Detects GFP in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant GFPuv Ser2-Lys238 Accession # P42212
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

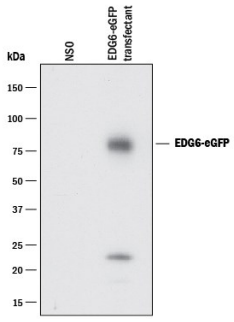
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	1-15 µg/mL	See Below
Immunoprecipitation	25 µg/mL	Conditioned cell culture medium spiked with Recombinant GFP, see our available Western blot detection antibodies
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Simple Western	2.5 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

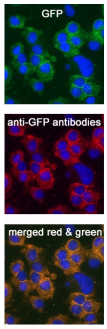
DATA

Western Blot



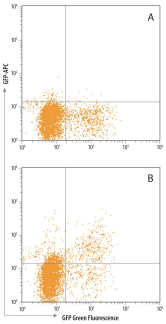
Detection of GFP by Western Blot. Western blot shows lysates of NS0 mouse myeloma cell line either mock transfected or transfected with eGFP-tagged EDG6. PVDF membrane was probed with 1 µg/mL of Goat Anti-GFP Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4240) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for GFP at approximately 80 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



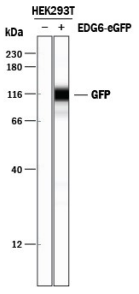
GFP in HEK293 human embryonic kidney cells transfected with GFP. GFP was detected in immersion fixed HEK293 human embryonic kidney cells transfected with GFP (green) using Goat Anti-GFP Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4240) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red, middle panel; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to the cytoplasm of GFP-positive cells. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of GFP in HEK293 human embryonic kidney cells transfected with GFP. HEK293 human embryonic kidney cells transfected with GFP was stained with Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody only (A, Catalog # F0108) or with Goat Anti-GFP Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4240) followed by Secondary Antibody (B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin. Quadrant markers were set based on control antibody staining (Catalog # AB-108-C).

Simple Western



Detection of GFP by Simple Western™. Simple Western lane view shows lysates of HEK293T human embryonic kidney cell line either mock transfected (-) or transfected with eGFP-tagged EDG6 (+), loaded at 0.2 mg/mL. A specific band was detected for GFP at approximately 114 kDa (as indicated) using 2.5 µg/mL of Goat Anti-GFP Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4240) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Green fluorescent protein (GFP) is a 27 kDa protein originally isolated from the jellyfish *Aequorea victoria*. In the presence of UV light (490-520 nm), it emits a green fluorescent color that can be used to pinpoint locations of various intracellular proteins. GFP is 238 amino acids (aa) in length. It is a globular monomer that has a tendency to dimerize. The monomer has the shape of a β-barrel with a chromophore (aa 65-67) containing α-helix running up its center. GFPuv is the *Aequorea* sequence with three aa substitutions; Phe to Ser at # 99, Met to Thr at # 153, and Val to Ala at # 163. This form expresses faster and is 18-fold brighter than native GFP; excitation peaks at 395 nm and emission at 508 nm.

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