

DESCRIPTION

Specificity	Stains eGFP transfectants but not irrelevant transfectants in flow cytometry. Detects recombinant GFPuv in Western blots. For optimal Western blot results, we recommend R&D Systems Goat Anti-GFP Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4240) or Novus Biologicals Mouse Anti-GFP Monoclonal Antibody (Catalog # NBP2-2211).
Source	Monoclonal Mouse IgG ₁ Clone # 454505
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -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.

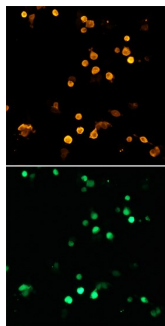
APPLICATIONS

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
Immunocytochemistry	8-25 µg/mL	See Below
Immunoprecipitation	2 µg/100 µg cell lysate	293T human embryonic kidney cell line transfected with eGFP
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

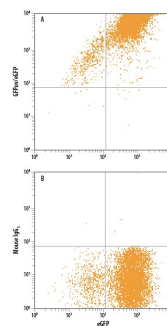
DATA

Immunocytochemistry



GFP was detected in 2% paraformaldehyde immersion fixed HEK293 human embryonic kidney cell line transfected with GFP-expressing protein using Mouse Anti-GFP Monoclonal Antibody (Catalog # MAB42401) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red, upper panel; Catalog # NL007). Lower panel shows transfected cells by GFPuv auto-fluorescence (green). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Intracellular Staining by Flow Cytometry



Detection of eGFP in HEK293 Human Cell Line Transfected with eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with eGFP was stained with and either (A) Mouse Anti-GFP Monoclonal Antibody (Catalog # MAB42401) or (B) Mouse IgG₁ Isotype Control (Catalog # MAB002) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for [Staining Intracellular Molecules](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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. <ul style="list-style-type: none"> • 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.