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Green fluorescent protein (GFP, EGFP) pAb epitope purified

Quality Control Certificate of Analysis
Catalogue No.:A010-pGFP-5
Unit Size: 5 µg
Lot No: 0412-02

Background: The green fluorescent protein (GFP) is composed of 238 amino acid residues (26.9kDa) and exhibits bright green fluorescence when exposed to ultraviolet blue light (Prendergast & Mann 1978). It has become a well established reporter for gene expression and marker for protein targeting (Tsien 1998). Subtle modifications have been made to the sequence of GFP improving its solubility and photostablility (eGFP) to allow acquisition of more complex data (FRET, FRAP). Moreover, subtle residue modifications have been engineered to alter the emission wavelength such as in CFP, YFP to facilitate duel labelling (Shaner *et al.,* 2006). Anti-GFP Ab is a polyclonal antibody raised in rabbit by immunisation with, and affinity purification against full length GFP (Faix et al., 2001). It produces exceptional staining for GFP, PA-GFP, EGFP, CFP and YFP in western blotting at a dilution of 1:10,000 and bioimaging applications at high dilutions, it has featured in an impressive number of publications.

Description: Lyophilised **Rabbit** polyclonal monoclonal Ab to GFP

Immunogen: recombinant GFP together with complete Freund's adjuvant

Antibody Isotype: IgG

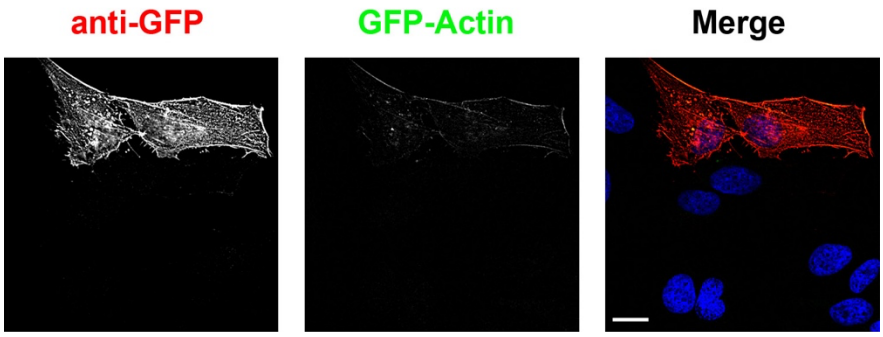
Antibody Purity: Affinity Purified using purified GFP covalently coupled to cyanogen bromide activated agarose

Vial Constituents: 5 µg of lyophilised Affinity purified A010-pGFP-5 in PBS + stabilizer.

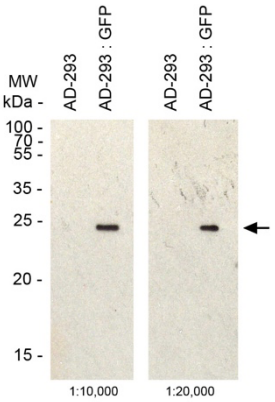
Storage Instructions: Lyophilised antibody is stable at 4 °C when stored with desiccant. Reconstitute lyophilised powder in **6.25 µl** of 18 MΩ H_2O, aliquot and store frozen at -80 °C for 1 year. Avoid freeze - thaw cycles.

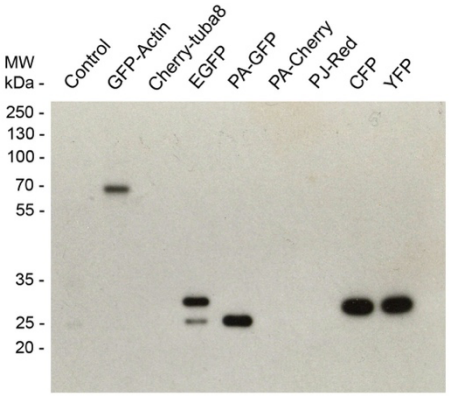
Tested Applications: **WB 1:10,000, IF 1:500-5000**, IP, ELISA

Specificity: A010-pGFP will recognise regular, enhanced and photo activatable green fluorescent proteins GFP, EGFP, PAGFP. It will also recognise cyan and yellow fluorescent proteins CFP, YFP, but will not recognise any red fluorescent proteins.



IF image showing the signal amplification of GFP-Actin using anti-GFP antibody (A010-pGFP). Hela cells with low level GFP-Actin expression were produced and fixed in PFA, permabilized using 0.1% Tween® 20 in PBS + 1% BSA and stained using 1:500 anti-GFP antibody for 1 hour in PBS + 1% BSA. Cells were washed and stained for 1 hour using 1:200 anti-Rabbit Alexa fluor 568, washed and mounted using mounting media with DAPI then imaged using an Olympus DeltaVision deconvolution microscope using a 40X (top) and 100X (bottom) lens. Scale bar 20 µm. We saw a dramatic improvement to the brightness and staining clarity in the red channel (anti-GFP) compared with the green channel (GFP-Actin low level expression).





WB against using 1:10,000 Rabbit anti-GFP Ab against various fluorescent proteins. Anti-GFP stains for GFP, EGFP, PA, GFP, CFP and YFP, but not RFP varients.

WB using A010-pGFP against AD-293 cells +/- GFP expression. AD-293 cells in a 2cm dish (6 well plate) were transfected with a GFP construct and incubated for 24 hours. Cells were harvested in 500 µl of SDS loading buffer and boiled. 10 µl loaded per lane.

Related Products: A010-pRFP-5 / 50

Background References:

Faix, J., Weber, I., Mintert, U., Köhler, J., Lottspeich, F., and Marriott, G. (2001) *EMBO J* **20**, 3705-3715
Prendergast, F. G., and Mann, K. G. (1978) *Biochemistry* **17**, 3448-3453
Shaner, N. C., Steinbach, P. A., and Tsien, R. Y. (2005) *Nat Methods* **2**, 905-909
Tsien, R. Y. (1998) *Annu Rev Biochem* **67**, 509-544