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AP09229PU-N Polyclonal Antibody to RFP-Tag (Ads. to Hu, Ms, Rt Serum Proteins) - Aff - Purified

Alternate names: DsRed Tag, Red fluorescent protein Tag

Quantity: 0.1 mg

Concentration: 0.95 mg/ml (by UV absorbance at 280 nm)

Background: Epitope tagging is a powerful and versatile strategy for detecting and purifying proteins expressed by cloned genes. To utilize this feature, protein expression vectors are typically engineered with a nucleotide sequence that encodes the peptide epitope tag. The gene of interest is cloned in-frame relative to the tag and, upon expression, the protein of interest is synthesized as a fusion protein with the peptide tag. Fusion protein detection and/or purification is mediated by highly specific antibodies to the engineered peptide, thus eliminating the need for antibodies to proteins from each newly cloned gene. Commonly used epitope tags include glutathione-S-transferase (GST), c-myc, 6-histidine (6X-His), FLAG, green fluorescent protein (GFP), red fluorescent protein (RFP, DSRed), maltose binding protein (MBP), influenza A virus haemagglutinin (HA), b-galactosidase, and GAL4.

Uniprot ID: Q9U6Y8

NCBI: 86600

Host / Isotype: Rabbit / IgG

Immunogen: Red Fluorescent Protein (RFP) fusion protein corresponding to the full length amino acid sequence (234aa) derived from the mushroom polyp coral Discosoma
Genename: DsRed

Format:
State: Liquid (sterile filtered) purified Ig fraction
Purification: Immunoaffinity Chromatography using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities
Buffer System: 0.02M Potassium Phosphate, 0.15M Sodium Chloride, pH 7.2
Preservatives: 0.01% (w/v) Sodium Azide
Stabilizers: None

Applications: Antibodies to RFP (Discosoma spp.) are intended for use in immunological assays including ELISA, Western blotting, Fluorometry and Fluorescence Activated Cell Sorting (FACS).
Polyclonal anti-RFP is designed to detect RFP and its variants. This antibody can be used to detect RFP by ELISA (Sandwich or Capture) for the direct binding of antigen. Biotin conjugated polyclonal anti-RFP used in a sandwich ELISA with unconjugated anti-RFP is well suited to titrate RFP in solution. The detection antibody conjugated to Biotin is subsequently reacted with streptavidin conjugated HRP.
Fluorochrome conjugated polyclonal anti-RFP can be used to detect RFP by Immunofluorescence microscopy in cell expression systems and can detect RFP containing inserts. Significant amplification of signal is achieved using fluorochrome conjugated polyclonal anti-RFP relative to the fluorescence of RFP alone.

For Immunoblotting use either Alkaline Phosphatase or Peroxidase conjugated polyclonal anti-RFP to detect RFP or RFP containing proteins on Western blots.
Recommended Dilutions:
ELISA: 1/20,000-1/50,000.
Immunofluorescence: 1/200-1/2,000.
Western blot: 1/1,000-1/5,000.
Immunohistochemistry: 1/200-1/2,000.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

Polyclonal anti-RFP is designed to detect RFP and its variants.
Assay by Immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum and purified and partially purified Red Fluorescent Protein (Discosoma). No reaction was observed against Human, Mouse or Rat serum proteins. ELISA was used to confirm specificity at less than 0.1% of target signal.
Expect reactivity against RFP and its variants: mCherry, tdTomato, mBanana, mOrange, mPlum, mOrange and mStrawberry.

Storage:

Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Dilute only prior to immediate use.
Centrifuge product if not completely clear after standing at room temperature.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Product Citations:

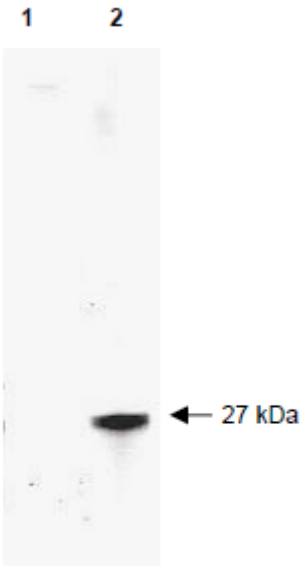
Originator or purchased from resellers:
1. Takeda N, Jain R, Leboeuf MR, Padmanabhan A, Wang Q, Li L, et al. Hopx expression defines a subset of multipotent hair follicle stem cells and a progenitor population primed to give rise to K6+ niche cells. Development. 2013 Apr;140(8):1655-64. doi: 10.1242/dev.093005. Epub 2013 Mar 13. PubMed PMID: 23487314.

General Readings:

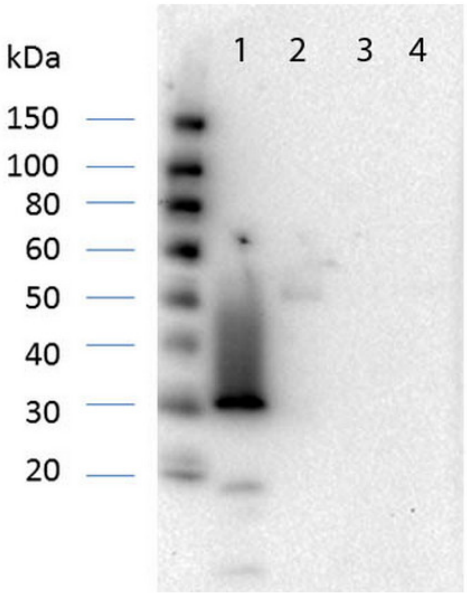
1. Gross LA, Baird GS, Hoffman RC, Baldridge KK, Tsien RY. The structure of the chromophore within DsRed, a red fluorescent protein from coral. Proc Natl Acad Sci U S A. 2000 Oct 24;97(22):11990-5. PubMed PMID: 11050230.

Pictures:

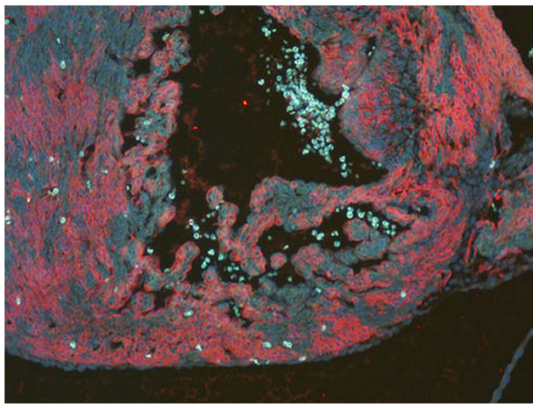
Western blot of RFP recombinant protein detected with polyclonal anti-RFP antibody. Lane 1 shows no reaction against a GFP recombinant protein present in 10 µg of HeLa cell extract. Lane 2 shows a single band detected in 10 µg of a HeLa lysate containing RFP recombinant protein as a 27 kDa band. A 4-12% Bis-Tris gradient gel was used for SDS-PAGE. The membrane was blocked and then probed with Anti-RFP diluted 1:2,500 for 1 h at RT followed by washes and reaction with a 1:5,000 dilution of IRDye(TM)800 conjugated Goat-a-Rabbit IgG [H&L]. IRDye(TM)800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



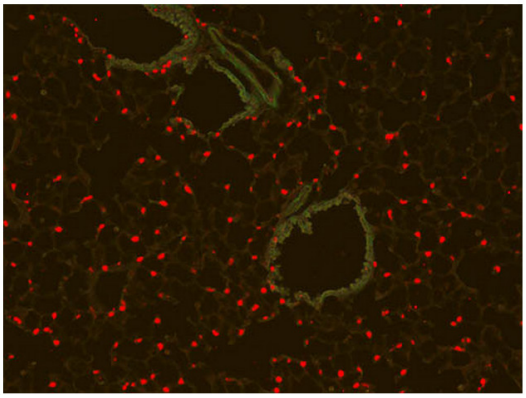
Western Blot of RFP Antibody Pre-Absorbed: Lane 1: RFP, Lane 2: Human IgG, Lane 3: Goat IgG, Lane 4: Mouse IgG Load: 50ng per lane. Primary antibody: RFP Antibody Pre-Absorbed at 1/1,000 overnight at 4°C. Secondary antibody: Peroxidase conjugated rabbit secondary antibody at 1/40,000 for 30 min at RT. Block: MB-070 Blocking Buffer for 30 min at RT. Predicted/Observed size: 27 kDa, 30 kDa



Immunofluorescence Microscopy of Rabbit Anti-RFP antibody. Tissue: (10X) Mouse E14.5 embryo heart tissue. Fixation: 4% PFA. Antigen retrieval: Heat. Primary antibody: Anti-RFP antibody at 1/50 for 1 h at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1/250 for 1 hr at RT. Staining: cardiac cells are RFP positive in red in tomato transgenic mice.



Immunofluorescence Microscopy of Rabbit Anti-RFP antibody. Tissue: (10X) Mouse lung tissue. Fixation: 4% PFA. Antigen retrieval: Heat. Primary antibody: Anti-RFP antibody at 1/50 for 1 h at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1/250 for 1 hr at RT. Staining: SPC+ cells are RFP positive in red.



Immunofluorescence Microscopy of Rabbit Anti-RFP antibody. Tissue: HopERCre/+; R26Tom/+ mice. Fixation: 0.5% PFA. Antigen retrieval: Tamoxifen. Primary antibody: RFP antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1/10,000 for 45 min at RT. Localization: RFP is nuclear and occasionally cytoplasmic. Staining: Hop-derived cells in the hair follicle, labeled in red.