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Datasheet for 600-402-379

RFP Antibody Fluorescein Conjugated Pre-Adsorbed

Overview

Description:	Anti-RFP (RABBIT) Antibody Fluorescein Conjugated (Min X Hu Ms & Rt Serum Proteins) - 600-402-379
Item No.:	600-402-379
Size:	100 µg
Applications:	Dot Blot, FISH, IF, IHC
Reactivity:	RFP, rRFP
Host Species:	Rabbit

Product Details

Background:	Fluorescent proteins such as Discosoma Red Fluorescent Protein (DsRed) from sea anemone Discosoma sp. mushroom or green fluorescent protein (GFP) from Aequorea victoria jellyfish are widely used in research practice. Fusion RFP and GFP commonly serve as marker for gene expression and protein localization. As DsRed and GFP share only 19% identity, therefore, in general, anti-GFP antibodies do not recognize DsRed protein and vice versa. Structurally, Discosoma red fluorescent protein is similar to Aequorea green fluorescent protein in terms of its overall fold (a β-can) and chromophore-formation chemistry. However, Discosoma red fluorescent protein undergoes an additional step in the chromophore maturation and obligates tetrameric structure. Rockland offers many controls, monoclonal, and polyclonal antibodies for RFP.
Synonyms:	rabbit anti-RFP antibody fluorescein conjugation, FITC conjugated rabbit anti-RFP antibody, DsRed, rDsRed, Discosoma sp. Red Fluorescent Protein, Red fluorescent protein drFP583
Host Species:	Rabbit
Conjugate:	Fluorescein (FITC)
Clonality:	Polyclonal
Format:	IgG
F/P Ratio:	3.4

Target Details

Gene Name:	DsRed

Reactivity:	RFP, rRFP
Immunogen Type:	Recombinant Protein
Immunogen:	The immunogen is a Red Fluorescent Protein (RFP) fusion protein corresponding to the full length amino acid sequence (234aa) derived from the mushroom polyp coral Discosoma.
Purity/Specificity:	This product was prepared from monospecific antiserum by immunoaffinity chromatography using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Expect reactivity against RFP and its variants: mCherry, tdTomato, mBanana, mOrange, mPlum, mOrange and mStrawberry. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-fluorescein, 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.
Relevant Links:	• UniProtKB - Q9U6Y8

Application Details

Tested Applications:	Dot Blot
Suggested Applications:	FISH, IF, IHC (Based on references)
Application Note:	Polyclonal anti-RFP is designed to detect RFP and its variants. This fluorescein conjugated antibody has been tested by dot blot and can be used to detect RFP by ELISA (sandwich or capture) for the direct binding of antigen. Significant amplification of signal is achieved using fluorochrome conjugated polyclonal anti-RFP relative to the fluorescence of RFP alone. Optimal titers for applications should be determined by the researcher.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
FLISA:	>1:20,000
IF:	1:500 - 1:2,500
WB:	>1:10,000

Formulation

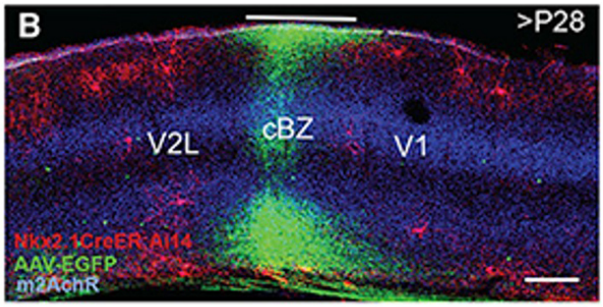
Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free

Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

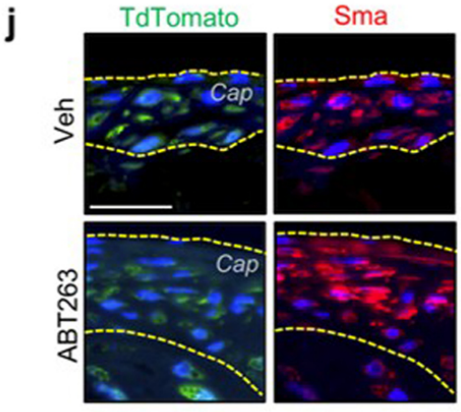
Shipping & Handling

Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

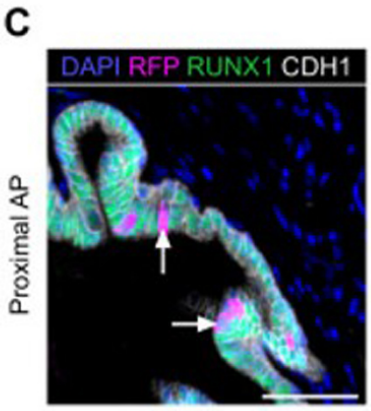
Images



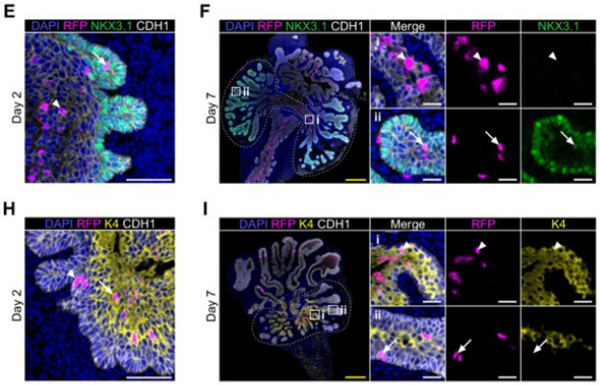
Immunofluorescence Microscopy
(B) Example AAV-EGFP-labeled callosal axon projection into contralateral V1/V2L border region where ChCs express RFP. The region that received callosal input is labeled as callosal binocular zone (cBZ). Fig 1. PMID: 33290732



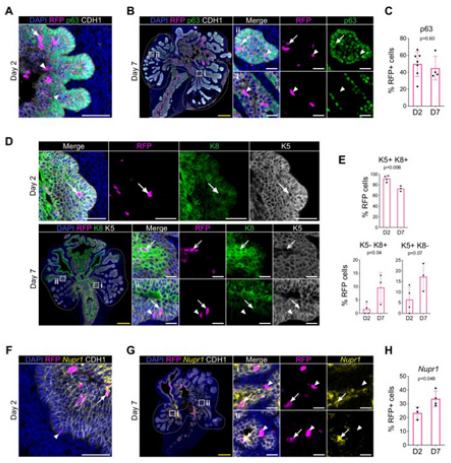
Immunofluorescence Microscopy
j, Representative images of tdTomato+/Sma+ cells in fibrous caps (bracketed in yellow dashed lines; quantified in k) from indicated groups. Fig 5. PMID: 34746803



Immunofluorescence Microscopy
(C) Co-immunostaining of RFP, RUNX1, CDH1 in the proximal AP. Arrows indicate RFP labeled RUNX1+ cells. Scale bar: 50 µm. Fig 4. PMID: 33025905



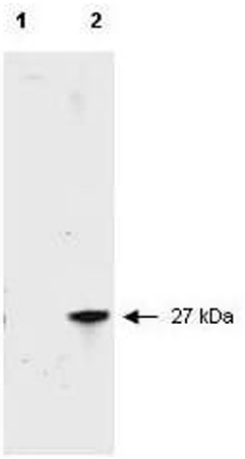
Immunofluorescence Microscopy
(E, F) Co-immunostaining of RFP, NKX3.1, CDH1 in UGS explants harvested at day 2 (E) and day 7 (F). Higher magnification images of (i) proximal and (ii) distal regions are shown for day 7. Arrows show RFP+ NKX3.1+ cells, arrowheads show RFP+ NKX3.1- cells. Scale bars: 200 µm (yellow) and 50 µm (white). (H, I) Co-immunostaining of RFP, K4, CDH1 in UGS explants harvested at day 2 (H) and day 7 (I). Higher magnification images of (i) proximal and (ii) distal regions are shown for day 7. Arrows show RFP+ K4 cells, arrowheads show RFP+ K4+ cells. Scale bars: 200 µm (yellow) and 50 µm (white). Fig 7. PMID: 33025905



Immunofluorescence Microscopy

(A, B) Co-immunostaining of RFP, p63, CDH1 in UGS explants harvested at day 2 (A) and day 7 (B). Higher magnification images of (i) proximal and (ii) distal regions are shown for day 7. Arrows show RFP+ p63+ cells, arrowheads show RFP + p63- cells. Scale bars: 200 µm (yellow) and 50 µm (white). (C) Quantification of the percentage of epithelial RFP + p63+ cells at day 2 (n = 7) and day 7 (n = 4) of UGS explant cultures. Quantification was performed within the boundaries delimited in B by dotted lines. (D) Co-immunostaining of RFP, K5, K8 in UGS explants harvested at day 2 (top) and day 7 (bottom). Higher magnification images of proximal (i) and (ii) distal regions are shown for day 7. Arrows show RFP+ K5+ K8+ cells, chevron arrows show RFP + K5- K8+, arrowheads show RFP+ K5+ K8- cells. Scale bars: 200 µm (yellow) and 50 µm (white). (E) Quantification of the percentage of epithelial K5+ K8+, K5- K8+ cells and K5+ K8- cells in the RFP subset at day 2 (n = 4) and day 7 (n = 3) of UGS explant cultures. Quantifications were performed within the boundaries delimited in D by dotted lines. (F, G) Co-immunostaining of RFP, Nupr1 (mRNA), CDH1 in UGS explants harvested at day 2 (F) and day 7 (G). Higher magnification images of (i) proximal and (ii) distal regions are shown for day 7. Arrows show RFP+ Nupr1+ cells, arrowheads show RFP+ Nupr1- cells. Scale bars: 200 µm (yellow) and 50 µm (white). (J) Quantification of the percentage of epithelial K4+ cells in the RFP subset at day 2 (n = 3) and day 7 (n = 4) of UGS explant cultures. Quantification was performed within the boundaries delimited in G by dotted lines. Source files are available in Figure 7—figure supplement 1—source data 1.

PMID: 33025905



Western Blot
Western blot of RFP recombinant protein detected with Rockland's unconjugated polyclonal anti-RFP antibody (600-401-379). Lane 1 shows no reaction against a GFP recombinant protein present in 10 ug of HeLa cell extract. Lane 2 shows a single band detected in 10 ug of a HeLa lysate containing RFP recombinant protein. Rockland's polyclonal anti-RFP detects a 27 kDa band corresponding to the epitope tag RFP. A 4-12% Bis-Tris gradient gel (Invitrogen) was used for SDS-PAGE. The protein was transferred to nitrocellulose using standard methods. After blocking the membrane was probed with the unconjugated primary antibody diluted 1:2,500 for 1 h at room temperature followed by washes and reaction with a 1:5,000 dilution of IRDye™800 conjugated Goat-a-Rabbit IgG [H&L] MX (611-132-122). IRDye™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.

References

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- Wang SB et al. Retinal and callosal activity-dependent chandelier cell elimination shapes binocularity in primary visual cortex. *Neuron.* (2021)
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- Mevel R et al. RUNX1 marks a luminal castration-resistant lineage established at the onset of prostate development. *Elife.* (2020)

Disclaimer

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