



Datasheet for ABIN129578

## anti-RFP antibody



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1 Validation

11 Images

355 Publications

### Overview

Quantity:	100 µL
Target:	RFP
Reactivity:	Discosoma
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This RFP antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunofluorescence (IF), Immunoprecipitation (IP), Immunohistochemistry (IHC), Flow Cytometry (FACS), Immunohistochemistry (Frozen Sections) (IHC (fro)), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))

### Product Details

Purpose:	Polyclonal RFP antibody is designed to detect RFP and its variants.
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. Immunogen Type: Recombinant Protein
Isotype:	IgG
Cross-Reactivity (Details):	Expect reactivity against RFP and its variants: mCherry, tdTomato, mBanana, mPlum, mOrange and mStrawberry. 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.
Purification:	This product was prepared from monospecific antiserum by immunoaffinity chromatography

## Product Details

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using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities.

Preadsorption: Pre-adsorbed

Sterility: Sterile filtered

## Target Details

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Target: RFP

Alternative Name: RFP ([RFP Products](#))

Background: Synonyms: DsRed, rDsRed, Discosoma sp. Red Fluorescent Protein, Red fluorescent protein drFP583

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  $\beta$ -can) and chromophore-formation chemistry. However, Discosoma red fluorescent protein undergoes an additional step in the chromophore maturation and obligates tetrameric structure.

## Application Details

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Application Notes: Suggested dilutions:

ELISA: 1:28,700 - 1:48,700

IF: 1:200 - 1:2,000

FACS: 1:200 - 1:2,000

WB: 1:1,000 - 1:5,000

IHC: 1:200 - 1:2,000

IP: User Optimized

Comment: 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

## Application Details

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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.

Optimal titers for applications should be determined by the researcher.

Restrictions: For Research Use only

## Handling

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Format: Liquid

Concentration: 1.0 mg/mL

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 0.01% (w/v) Sodium Azide

Preservative: Sodium azide

Precaution of Use: This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Handling Advice: Avoid cycles of freezing and thawing.

Storage: 4 °C/-20 °C

Storage Comment: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. 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.

Expiry Date: 12 months

## Publications

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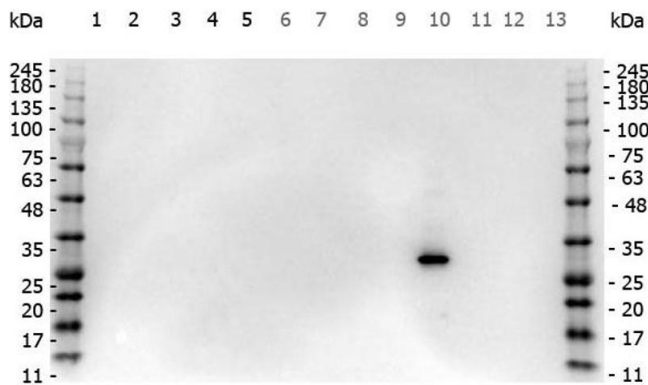
Product cited in: Daniel, Russ, Guthridge, Raina, Estes, Parsons, Richardson, Schroeder, Zarnescu: "miR-9a mediates the role of Lethal giant larvae as an epithelial growth inhibitor in Drosophila." in: **Biology open**, Vol. 7, Issue 1, (2018) ([PubMed](#)).

Bankoti, Ogawa, Nguyen, Emadi, Couse, Salehi, Fan, Dhall, Wang, Brown, Funari, Tang, Martins: "Differential regulation of Effector and Regulatory T cell function by Blimp1." in: **Scientific reports**, Vol. 7, Issue 1, pp. 12078, (2017) ([PubMed](#)).

Guo, Maeda, Ma, Delgado, Sohn, Miers, Ko, Bannerman, Xu, Wang, Zhou, Takebayashi, Pleasure: "Macroglial plasticity and the origins of reactive astroglia in experimental autoimmune encephalomyelitis." in: **The Journal of neuroscience : the official journal of the Society for Neuroscience**, Vol. 31, Issue 33, pp. 11914-28, (2011) ([PubMed](#)).

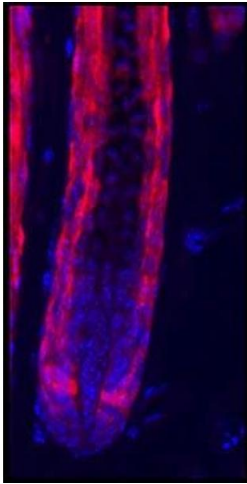
There are more publications referencing this product on: [Product page](#)

Images



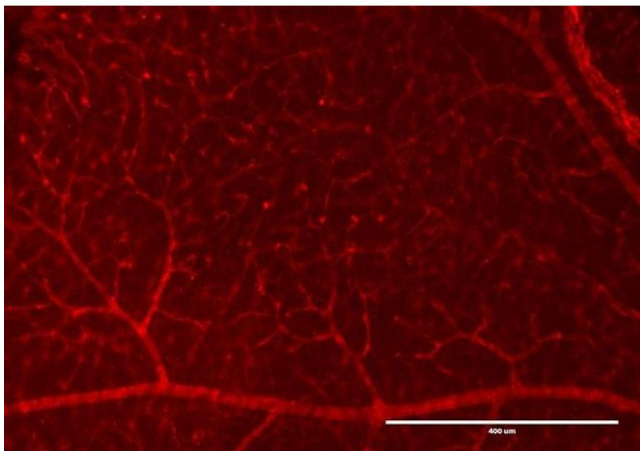
Western Blotting

**Image 1.** Western Blot of Rabbit anti-RFP antibody. Marker: Opal Pre-stained ladder. Lane 1: HEK293 lysate (ABIN964025). Lane 2: HeLa Lysate (ABIN964023). Lane 3: CHO/K1 Lysate. Lane 4: MDA-MB-231. Lane 5: A431 Lysate (ABIN964021). Lane 6: Jurkat Lysate (ABIN964029). Lane 7: NIH/3T3 Lysate (ABIN964043). Lane 8: E-coli HCP Control (ABIN1607597). Lane 9: FLAG Positive Control Lysate (ABIN1607004). Lane 10: Red Fluorescent Protein (ABIN964533). Lane 11: Green Fluorescent Protein (ABIN964156). Lane 12: Glutathione-S-Transferase Protein. Lane 13: Maltose Binding Protein (ABIN964518). Load: 10  $\mu$ g of lysate or 50 ng of purified protein per lane. Primary antibody: RFP antibody at 1  $\mu$ g/mL overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (ABIN102010) at 1:30,000 for 60 min at RT. Blocking Buffer: 1 % Casein-TTBS for 30 min at RT. Predicted/Observed size: 30 kDa for RFP.



### Immunofluorescence

**Image 2.** 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.



### Immunofluorescence

**Image 3.** Immunofluorescence Microscopy of Rabbit Anti-RFP antibody. Tissue: DsRed transgenic mouse retina. Fixation: 4 % PFA. Blocking: 3 % BSA, 0.3 % Triton Primary antibody: RFP antibody at 1:100 for 12 h at 4 °C. Secondary antibody: Alexa488 secondary antibody at 1:10,000 for 4 hours at RT. Localization: RFP is nuclear and occasionally cytoplasmic. Staining: labeled in red.

Please check the [product details page](#) for more images. Overall 11 images are available for ABIN129578.



### Successfully validated (Immunofluorescence (IF))

by [Centre d'Immunologie de Marseille-Luminy](#)

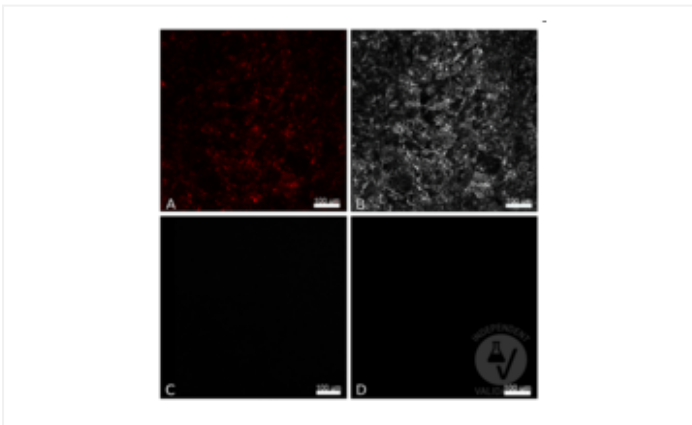
Report Number: 101103

Date: Mar 07 2017

Target:	RFP
Lot Number:	34945
Method validated:	Immunofluorescence (IF)
Positive Control:	CX3CL1mcherry <sup>+</sup> murine thymus
Negative Control:	CX3CL1 WT murine thymus
Notes:	Passed. The RFP antibody ABIN129578 specifically labels RFP <sup>+</sup> cells in CX3CL1mcherry <sup>+</sup> murine thymus in immunofluorescence, consistent with the expression pattern of CX3CL1mcherry.
Primary Antibody:	ABIN1043867
Secondary Antibody:	donkey Fab'2 anti-rabbit A647 conjugated antibody (Jackson Immunoresearch, 711-606-152, lot 128806)
Protocol:	<ul style="list-style-type: none"><li>• Harvest thymus from 7 weeks old mice in 1X Dulbecco's phosphate buffered saline (DPBS) (Gibco Life Technologies, 14200-067).</li><li>• Fix mouse thymus in antigen fix (Diapath, P0014) for 2h at 4°C.</li><li>• Wash tissue in 0.1M pH7.4 phosphate buffer at for 1h at 4°C.</li><li>• Dehydrate tissue in 30% sucrose solution at 4°C ON.</li><li>• Snap freeze tissue in Tissue Freezing Medium (ElectroMicroscopy Science, 72592-C) at -80°C.</li><li>• Cut blocks into 25µm sections using a cryostat (Leica, CM3050 S).</li><li>• Transfer sections to a slide.</li><li>• Create a hydrophobic barrier on the slide around sections with Dako pen (Dako, S2002, lot 00081640).</li><li>• Place slide in a humidified chamber and rehydrate sections in 0.1 M TrisHCl pH7.4 for 10min at RT.</li><li>• Gently remove buffer by tapping slide.</li><li>• Permeabilize tissue in 0.1M TrisHCl pH7.4 containing 2% Triton X-100 (Sigma, lot 015K0039) and 0.5% BSA, for 20min at RT.</li><li>• Incubate sections with primary RFP antibody (Red Fluorescent Protein) (AA 234) (antibodies-online, ABIN129578, lot 34945) diluted 1:1000 0.1M TrisHCl pH7.4 containing 2% Triton X-</li></ul>

- 100 (Sigma, batch 015K0039) and 0.5% BSA for 2h at RT.
- Incubate a no primary antibody negative control in parallel in 0.1M TrisHCl pH7.4 containing 2% Triton X-100 (Sigma, batch 015K0039) and 0.5% BSA.
  - Wash slides 1x 5min in 0.1M TrisHCl pH7.4.
  - Incubate sections with secondary donkey Fab'2 anti-rabbit AF647 conjugated antibody (Jackson ImmunoResearch, 711-606-152, lot 128806) diluted 1:300 in 0.1M TrisHCl pH7.4 containing 2% Triton X-100 (Sigma, batch 015K0039) and 0.5% BSA for 2h at RT.
  - Wash slides 1x 5min in 0.1M TrisHCl pH7.4.
  - Add approximately 10 $\mu$ L of Slowfade Gold antifade reagent (ThermoFisher Scientific, S36937, lot 1226836) for each section and mount cover slip.
  - Image acquisition on an LSM 880 (Zeiss), 20x magnification, 1000 resolution.

## Image for Validation report #101103



### Validation image no. 1 for anti-Red Fluorescent Protein (RFP) antibody (ABIN1043867)

CX3CL1 positive cells in CX3CL1 mcherry murine thymus (A, mCherry) were stained with ABIN1043867 and an AF647 conjugated secondary antibody (B) or with the secondary antibody alone (C). CX3CL1 positive cells in CX3CL1 wt murine thymus were stained with ABIN1043867 and the AF647 conjugated antibody (D). Scale bar 100 $\mu$ m, 20x magnification, 1000 resolution