

Datasheet for 400-001-MS3**Recombinant RFP Antibody****Overview**

Description:	Anti-RFP (VHH) Single Domain Antibody - 400-001-MS3
Item No.:	400-001-MS3
Size:	50 µg
Applications:	Dot Blot, ELISA, WB
Reactivity:	RFP
Origin:	Llama
Expressed in:	E. coli

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, polyclonal and recombinant antibodies for RFP.

A single-domain antibody (sdAb) is a small antibody fragment consisting of the monomeric variable domain derived from camelid heavy chain-only immunoglobulins naturally found in llamas, alpacas and camels. Also known as VHH antibodies, these are the smallest functional antigen-binding fragment that occurs in nature (12 - 14 kDa) and are now being used in biotechnology as a novel antibody scaffold. The small size of the VHH single domain antibody makes it very attractive for use in diagnostic imaging and potentially therapeutic applications.

Synonyms:	recombinant anti-RFP antibody, Red Fluorescent Protein antibody, DsRed, rDsRed, Discosoma sp. Red Fluorescent Protein, Red fluorescent protein drFP583
Species of Origin:	Llama
Expressed in:	E. coli
Clonality:	Recombinant Monoclonal

Clone ID: RFP40

Target Details

Gene Name:	DsRed
Reactivity:	RFP
Purity/Specificity:	Anti-RFP (VHH) Antibody is a recombinant antibody. The clone was isolated from a library prepared from a hyper-immunized llama host and purified by affinity chromatography from bacterial culture. Expect reactivity against RFP-labeled probes and proteins.
Relevant Links:	UniProtKB - Q9U6Y8

Application Details

Tested Applications:	Dot Blot, ELISA, WB
Application Note:	Anti-RFP is a his-tagged monoclonal recombinant antibody designed to detect Red Fluorescent Protein and its conjugates. This antibody has been tested by western blot, dot blot, and ELISA and is intended for use in immunological assays including immunofluorescence and fluorescence activated cell sorting (FACS). The antibody can be labeled with dyes, enzymes or fluorescence, directly or secondarily, for visualization and detection of RFP-conjugated molecules by immunofluorescence. Secondary detection can be achieved using conjugated anti-His tag or anti-VHH antibodies. Optimal titers for applications should be confirmed by the end user. This antibody is not suitable for Western blot detection of denatured RFP.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:31,000
WB:	1:1000

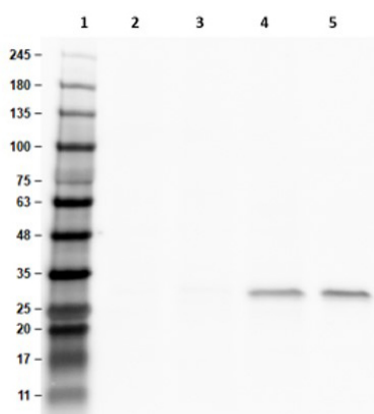
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by BCA assay
Buffer:	1X PBS, pH 7.4
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	20% (v/v) Glycerol

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. 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



Western Blot

Western Blot Results of Anti-RFP (VHH) Single Domain Antibody.

Lane 1: Opal Prestained Molecular Weight (p/n MB-210-0500).

Lane 2: HEK293 Whole Cell Lysate (p/n W09-000-365) [10ug] (-).

Lane 3: HEK293 Whole Cell Lysate (p/n W09-000-365) [10ug] + GFP rec. protein (p/n 000-001-215) [1ug] (-).

Lane 4: HEK293 Whole Cell Lysate (p/n W09-000-365) [10ug] + RFP rec. protein (p/n 000-001-379) [1ug] (+).

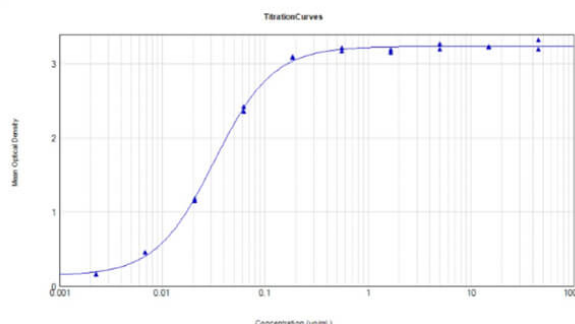
Lane 5: RFP rec. protein (p/n 000-001-379) [1ug] (+).

Primary Antibody: Anti-RFP (VHH) Single Domain Antibody (p/n 400-001-MS3) at 1:1000 for overnight at 4°C.

Secondary Antibody: Mouse Anti-6x His DL649 conjugated (p/n 200-343-382) at 1:10,000 for 1hr at RT.

Block: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) overnight at 4°C.

Expected MW: ~27kDa for RFP protein.

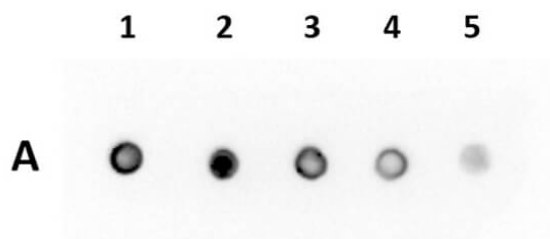


ELISA

ELISA Results of purified recombinant Anti-RFP Single Domain Antibody reactive against RFP. The working dilution for clone RFP40 is 1:31,000. Each well was coated in duplicate with 1.0µg of RFP (p/n 000-001-379). The starting dilution of antibody was 45µg/mL and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% Fish Gel/PBS blocking buffer (p/n MB-066), Mouse Anti-FLAG Tag HRP conjugated Antibody (p/n 200-303-383) and TMB substrate (p/n TMBE-1000).

Dot Blot

Dot Blot of Anti-RFP Single Domain Antibody. Row A: Recombinant RFP protein (p/n 000-001-379). Amount Dotted: (1) 200, (2) 100, (3) 50, (4) 25, (5) 12.5ng. Primary Antibody: Anti-RFP (VhH) 1.0µg/mL for 1.0hr at RT. Secondary Antibody: Mouse Anti-Flag Peroxidase (p/n 200-303-383) at 1:5000 for 30 mins at RT. Block: BlockOut Buffer (p/n MB-073). Exposure: 10 seconds.



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