ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Anti-Red Fluorescent Protein (RFP/dsRed) Antibody and controls

Ì **Cat.** RFP12-M	Monoclonal Anti-Red Fluorescent Proteins/Tag (RFP-tag) IgG	**SIZE**: 100 ul
Ì **Cat.** RFP12-C	Recombinant purified Red Fluorescent Proteins protein control for Western blot	**SIZE**: 100 ul

Recombinant DNA technology allows the addition of short pieces of well-defined tags, "peptides" or proteins at the amino or c-terminus of target genes, which can provide 'affinity handles' designed to bind specific matrices. Therefore, tags enables a selective identification and purification of the protein of interest. The addition of a green fluorescent protein (GFP) tag or red fluorescent protein (RFP) or yellow fluorescent protein (YFP) to a given gene, creates a stable fusion product that does not appear to interfere with the bioactivity of the protein, or with the biodistribution of the fluorescent tagged product.

Red **fluorescent protein (RFP)** is a common and useful biological marker for monitoring physiological processes, visualizing protein localization, and detecting transgenic expression in vivo. RFP can be excited by the 488 nm or 532 nm laser line and is optimally detected at 588 nm. For example, these proteins have been used as markers of gene expression, expressed as fusions to track endogenous protein within cells, and applied with other fluorescent proteins (FPs) for use in FRET experiments. The availability of monomeric versions of these proteins has bolstered their worth as fusion tags and vaulted them into routine experimental use. The red fluorescence displayed by these proteins arises from the presence of an acylimine group conjugated with the standard p-hydroxybenzylideneimidazolinone GFP chromophore. The longer emission wavelength of RFPs makes them attractive for whole-animal imaging because cells are more transparent to red light. For imaging applications, higher emission wavelengths (650–900 nm) are desirable because they tend to minimize background absorption and light scattering by tissue components and are less damaging to cells, enabling longer acquisition times.

Naturally-occurring Anthozoa RFPs, such as zRFP574, eqFP578, DsRed, and eqFP611, are obligate oligomers that display emission wavelengths ranging from 574-611 nm. Several far-red (λem > 630 nm) monomeric RFPs such as mPlum, mKate2, and mNeptune monomeric RFPs have been developed using random mutagenesis.

Source of Antigen and Antibodies

Antigen	RFP peptides form the Discosoma sp. (sea anemone) N-terminal peptide-KLH conjugates
Antibody host/type	Mouse, monoclonal IgG1 (Cat # RFP12-M) supplied in PBS, pH 7.2 and 0.2% BSA, and 0.01% thimerosal
2-ab	Goat Anti-mouse IgG-HRP conjugate Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve control	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

#RFP12-C, RFC Western control

RFP was expressed and purified from E. coli (>98%, ~27 kda). For Western blot +ve control (**Cat # RFP11-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **YFP11-C** for good visibility with antibody Cat # **RFP12-M**. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **RFP11-C** solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly.

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG

☐ 100ul ☐ solution ☐ lyophilized powder

Supplied in **Buffer**: PBS, **Reconstitute powder** in Water

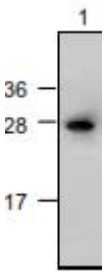
Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Recommended Usage



Western Blotting (1:200-1:1000)using Chemiluminescence technique. RFP Mol wt ~27 kda

ELISA (1:1000-1:5,000; using 50-100 ng of control peptide/well).

IP: 1:100-1:500

Specificity & Cross-reactivity

Antibody reacts with native and denatured forms of RFP and its variants: TagRFP, TurboRFP, dsRed, mCherry, mOrange, tdTomato, etc. Purified RFP protein (#RFP15-R) and western control (#RFP11-C) can be used as positive control

General References: Chica RA (2010) PNAS 107, 20257-20262; jach G (2001) Plant J. 28, 483-491; Mizuno H (2001) Biochemistry 40:2502–2510; Campbell RE (2002) Proc Natl Acad Sci USA 99:7877–7882; Shaner NC (2004) Nat Biotechnol. ;22:1567–1572; Pletneva N, et al. (2006) Acta Crystallogr D 62:527–532; Baird GS (2000) Proc Natl Acad Sci USA 97:11984–11989

*This product is for In vitro research use only.

Related material available from ADI

Catalog# ProdDescription

800-420-GFP Green Fluorescent Protein (GFP-fusion protein) ELISA Kit,
EGFP16-R Enhanced Green Fluorescent Proteins (EGFP) protein for ELISA EGFP16-R-100 Enhanced Green Fluorescent Proteins (EGFP) protein for ELISA

GFP11-A Anti-Green Fluorescent Proteins (GFP, A. victoria) protein, IgG
GFP11-HRP Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-HRP
GFP12-M Monoclonal Anti-Green Fluorescent Proteins (GFP, A. victoria) IgG
GFP15-R Green Fluorescent Proteins (GFP) protein for ELISA or Standards
GFP15-R-100 Green Fluorescent Proteins (GFP) protein for ELISA

RFP12-M Monoclonal Anti-Red Fluorescent Proteins/Tag (RFP-tag; Discosoma sea anemomone) IgG
RFP15-R Recombinant (E. coli) Red Fluorescent Proteins (RFP/dsRed) protein for ELISA or Standards (>98%)

YFP11-A Anti-Yellow Fluorescent Proteins (YFP) protein IgG
YFP11-C Recombinant (E. coli) Yellow Fluorescent Proteins (YFP) protein control for Western blot

RFP12-M-Monoclonal-Anti-RFP-IgG 150714A

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