Product Manual

RFP ELISA Kit

Catalog Number

AKR-122 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Red fluorescent protein (DsRed) is a spontaneously fluorescent protein isolated from the Indo-Pacific Sea coral, *Discosoma striata.* It absorbs and emits orange-red light and is well suited for multi-color tagging used in FRET. Since the molecular cloning of RFP cDNA and demonstration of RFP as a functional transgene, RFP has become a powerful tool with exciting applications in developmental, cell and molecular biology. RFP fluorescence is not species specific and can be expressed in bacteria, yeast, plant and mammalian cells. RFP can fuse with proteins of interest without interfering significantly with their assembly and function. Based on the structure of the RFP molecule, many RFP variants have been created with much improved fluorescence emission, or shifted excitation or emission spectra that are well suited for fluorescence microscopy and flow cytometry. Although RFP expression can be easily detected under a fluorescence microscope, RFP fluorescence intensity varies from cell to cell because of the heterogeneity nature of RFP expression. In order to quantitate the RFP expression in cells, FACS analysis is usually required, which is both expensive and time consuming.

Cell Biolabs' RFP ELISA Kit is an enzyme immunoassay developed for detection and quantitation of RFP or RFP fusion protein in cell or tissue samples. The quantity of RFP or its variants (including TagRFP, TurboRFP, DsRed, tdTomato, mCherry, mKate, mRuby, mBanana, mOrange, mPlum, and mStrawberry) in an unknown sample is determined by comparing its absorbance with that of a known recombinant RFP standard curve. The kit has detection sensitivity limit of 150 pg/mL RFP. The kit also provides an efficient system for rapid quantitation of RFP lentivirus titer for both viral supernatant and purified virus. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and RFP samples.

Related Products

1. AKR-120: GFP Quantitation Kit, Fluorometric
2. AKR-121: GPF ELISA Kit
3. STA-201: Recombinant EGFP

Kit Components

Box 1 (shipped at room temperature)

1. Anti-RFP Antibody Coated Plate (Part No. 212201): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-RFP Antibody (1000X) (Part No. 212202): One 15 µL vial of biotinylated antibody recognizing sea anemone *Discosoma* RFP and its variants.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant RFP Standard (Part No. 212203): One 100 µL vial of 2.5 µg/mL recombinant RFP in TBS plus BSA.

Materials Not Supplied

1. RFP Sample: cell or tissue lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store recombinant RFP Standard at -80ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-RFP Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-RFP antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of RFP Standard

Prepare a dilution series of recombinant RFP standards in the concentration range of 0 ng/mL to 10 ng/mL in Assay Diluent (Table 1).

Standard Tubes	2.5 µg/mL Recombinant RFP Standard (µL)	Assay Diluent (µL)	RFP (pg/mL)
1	4	996	10000
2	500 of Tube #1	500	5000
3	500 of Tube #2	500	2500
4	500 of Tube #3	500	1250
5	500 of Tube #4	500	625
6	500 of Tube #5	500	312
7	500 of Tube #6	500	156
8	0	500	0

Table 1. Preparation of samples for RFP Standard Curve

Assay Protocol

1. Prepare cell or tissue lysates containing RFP or RFP fusion protein.
   Note: Because the ELISA kit has a linear range of 156 pg/mL to 10000 pg/mL, we recommend using assay diluent to make series of 2-fold dilutions for each unknown sample.
2. Add 100 µL of RFP sample or RFP standard to the Anti-RFP Antibody Coated Plate. Each RFP sample, RFP standard and blank should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 2 hours on an orbital shaker.

4. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-RFP antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash the strip wells 5 times according to step 4 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 5 times according to step 4 above. Proceed immediately to the next step.
9. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the RFP ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

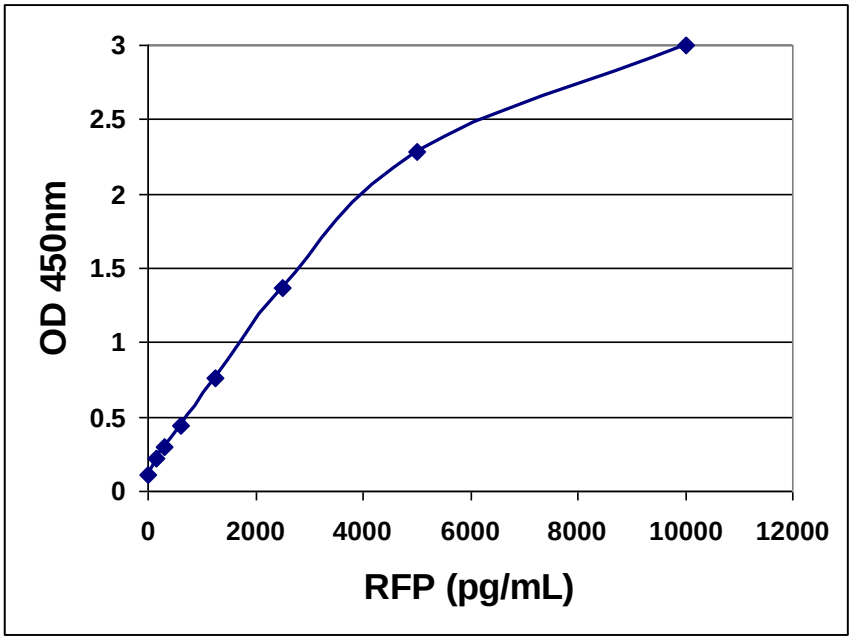

Figure 1: RFP ELISA Standard Curve.

Recent citation

Fang, J. et al. (2015). COPII dependent ER export: a critical component of insulin biogenesis and beta cell ER homeostasis. *Mol Endocrinol.* doi:10.1210/me.2015-1012.

References

1. Shaner, N.C. et al, *J. Cell Sci.* **120**: 4247-4260, 2007.
2. Matz, M.V. et al, *Nat. Biotechnol.* **17**: 969-973, 1999.
3. Campbell, R.E. et al, *Proc. Natl. Acad. Sci. USA* **99**: 7877-7882, 2002.
4. Shaner, N.C. et al, *Nat. Biotechnol*. **22**: 1567-1572, 2004.

Recent Product Citations

1. Popowski, K.D. et al. (2022). Inhalable dry powder mRNA vaccines based on extracellular vesicles. *Matter*. doi: 10.1016/j.matt.2022.06.012.
2. Zuniga, G. et al. (2022). Tau-induced deficits in nonsense-mediated mRNA decay contribute to neurodegeneration. *Alzheimers Dement*. doi: 10.1002/alz.12653.
3. Fang, J. et al. (2015). COPII dependent ER export: a critical component of insulin biogenesis and beta cell ER homeostasis. *Mol Endocrinol*. doi:10.1210/me.2015-1012.

Warranty

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