Product Manual

GFP ELISA Kit

Catalog Number

AKR-121	96 assays
AKR-121-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Green fluorescent protein (GFP) is a spontaneously fluorescent protein isolated from the pacific jellyfish, *Aequorea victoria.* It transduces the blue chemiluminescence into green fluorescent light. Since the molecular cloning of GFP cDNA and demonstration of GFP as a functional transgene, GFP has become a powerful tool with exciting applications in developmental, cell and molecular biology. GFP fluorescence is not species specific and can be expressed in bacteria, yeast, plant and mammalian cells. GFP can fuse with proteins of interest without interfering significantly with their assembly and function. Based on the structure of the GFP molecule, many GFP 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 GFP expression can be easily detected under a fluorescence microscope, GFP fluorescence intensity varies from cell to cell because of the heterogeneity nature of GFP expression. In order to quantitate the GFP expression in cells, FACS analysis is usually required, which is both expensive and time consuming.

Cell Biolabs' GFP ELISA Kit is an enzyme immunoassay developed for detection and quantitation of GFP or GFP fusion protein in cell or tissue samples. The quantity of GFP or its variants (including BFP, CFP and YFP) in an unknown sample is determined by comparing its absorbance with that of a known recombinant GFP standard curve. The kit has detection sensitivity limit of 30 pg/mL GFP. The kit also provides an efficient system for rapid quantitation of GFP lentivirus titer for both viral supernatant and purified virus. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and GFP samples.

Related Products

1. AKR-120: GFP Quantitation Kit, Fluorometric
2. AKR-122: RFP ELISA Kit
3. STA-201: Recombinant EGFP

Kit Components

Box 1 (shipped at room temperature)

1. Anti-GFP Antibody Coated Plate (Part No. 212101): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-GFP Antibody (1000X) (Part No. 212104): One 10 µL vial of biotinylated antibody recognizing jellyfish *Aequorea Victoria* GFP 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 GFP Standard (Part No. 212103): One 100 µL vial of 0.5 µg/mL recombinant GFP in TBS plus BSA.

Materials Not Supplied

1. GFP Sample: cell or tissue lysate
2. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store recombinant GFP 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-GFP Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-GFP antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:2000 with Assay Diluent. Do not store diluted solutions.

Preparation of GFP Standard

Prepare a dilution series of recombinant GFP standards in the concentration range of 0 ng/mL to 2 ng/mL in Assay Diluent (Table 1).

Standard Tubes	0.5 µg/mL Recombinant GFP Standard (µL)	Assay Diluent (µL)	GFP (pg/mL)
1	4	996	2000
2	500 of Tube #1	500	1000
3	500 of Tube #2	500	500
4	500 of Tube #3	500	250
5	500 of Tube #4	500	125
6	500 of Tube #5	500	62.5
7	500 of Tube #6	500	31.2
8	0	500	0

Table 1. Preparation of samples for GFP Standard Curve

Assay Protocol

1. Prepare cell or tissue lysates containing GFP or GFP fusion protein.
   Note: Because the ELISA kit has a linear range of 30 pg/mL to 2 ng/mL, we recommend using assay diluent to make series of 2-fold dilutions for each unknown sample.
2. Add 100 µL of GFP sample or GFP standard to the Anti-GFP Antibody Coated Plate. Each GFP sample, GFP standard and blank should be assayed in duplicate.

3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
4. Wash microwell strips 3 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-GFP antibody to each well.
6. Incubate at room temperature for 2 hours on an orbital shaker.
7. Wash the strip wells 3 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to all wells.
9. Incubate at room temperature for 1 hour on an orbital shaker.
10. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
11. 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.
12. 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).
13. 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 GFP ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

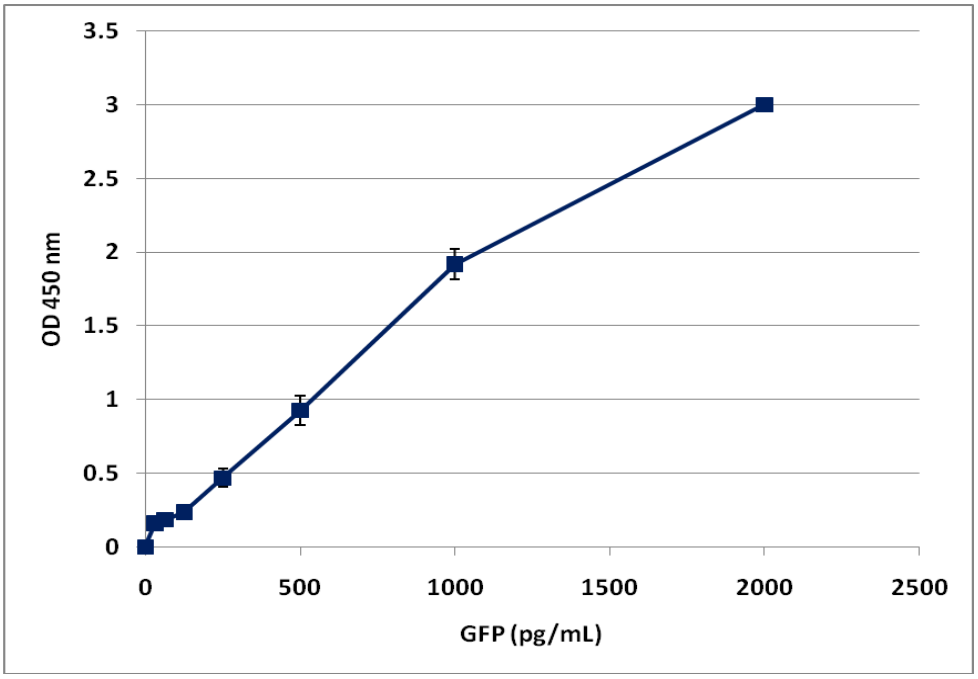

Figure 1: GFP ELISA Standard Curve.

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Recent Product Citations

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