Product Manual

Fluorescein Competitive ELISA Kit

Catalog Number

AKR-5141 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Fluorescein is a dye often used as a fluorescent tracer. It has a 494 nm absorption maximum and an emission maximum of 512 nm in water. Fluorescein isothiocyanate (FITC) and 6-FAM phosphoramidite (the latter used in oligonucleotide synthesis) are the two major derivatives of fluorescein.

FITC is commonly used to attach to biologically active molecules such as antibodies. These conjugates are used to label and track cells in fluorescence microscopy applications such as flow cytometry or immunofluorescent staining. Fluorescein can also be conjugated to nucleoside triphosphates and incorporated into a DNA probe enzymatically for in situ hybridization experiments. Sodium fluorescein is also employed to diagnose aberrations in the field of ophthalmology and optometry, where topical fluorescein can elucidate corneal abrasions, corneal ulcers and herpetic corneal infections. Fluorescein can also be used in rigid gas permeable contact lens fittings to evaluate the tear layer under the lens.

Cell Biolabs' Fluorescein Competitive ELISA Kit provides a convenient method for the detection of total fluorescein in extracts from cells, tissue, serum, plasma, or foods. The total content of fluorescein in unknown samples is determined by comparison with a fluorescein standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples. The kit has a detection sensitivity limit of 20 pM fluorescein.

Assay Principle

First, a fluorescein conjugate is coated on an ELISA plate. The unknown fluorescein samples or fluorescein standards are then added to the fluorescein conjugate preabsorbed ELISA plate. After a brief incubation, an anti-fluorescein antibody is added, followed by an HRP conjugated secondary antibody. The total content of fluorescein (or FITC) in unknown extracted samples is determined by comparison with a fluorescein standard curve.

Related Products

1. AKR-120: GFP Quantitation Kit, Fluorometric
2. AKR-121: GFP ELISA Kit
3. AKR-122: RFP ELISA Kit
4. AKR-130: His-Tag Protein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. HRP-Conjugated Anti-Fluorescein Monoclonal Antibody (Part No. 51411C): One 10 µL vial.
3. Assay Diluent (Part No. 310804): One 50 mL bottle.
4. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.

5. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
6. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Fluorescein Standard (Part No. 51412C): One 50 µL vial of 8 µM sodium fluorescein.
2. Fluorescein Conjugate (500X) (Part No. 51413C): One 25 µL vial.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. 1X PBS
2. Bovine Serum Albumin (BSA)
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Storage

Upon receipt, store the HRP Conjugated Anti-Fluorescein Antibody, Fluorescein Standard, Fluorescein Conjugate, and 100X Conjugate Diluent at -20ºC. Store all remaining components at 4°C.

Preparation of Reagents

- Fluorescein Conjugate Coated Plate:

  Note: The Fluorescein Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

  1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
  2. Immediately before use, prepare 1X Fluorescein Conjugate by diluting the 500X Fluorescein Conjugate in 1X Conjugate Diluent. Example: Add 10 µL of 500X Fluorescein Conjugate to 4.99 mL of 1X Conjugate Diluent.
  3. Add 100 µL of the 1X Fluorescein Conjugate to each well to be tested and incubate overnight at 4ºC. Remove the Fluorescein Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

- Anti-Fluorescein Antibody and Secondary Antibody: Immediately before use, dilute the Anti-Fluorescein antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Fluorescein standards in the concentration range of 0 to 80 nM by diluting the Fluorescein Standard in Assay Diluent (Table 1).

Standard Tubes	8 µM Fluorescein Standard (µL)	Assay Diluent (µL)	Fluorescein (nM)
1	5	495	80
2	100 of Tube #1	300	20
3	100 of Tube #2	300	5
4	100 of Tube #3	300	1.25
5	100 of Tube #4	300	0.313
6	100 of Tube #5	300	0.078
7	100 of Tube #6	300	0.020
8	0	300	0

Table 1. Preparation of Fluorescein Standards

Preparation of Samples

- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- Cells or tissues: Homogenize 50-200 mg of the cell pellet or tissue in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder. Perform dilutions in Assay Diluent as necessary.
- Food samples: Homogenize 1-5 grams using a mortar and pestle or by dounce homogenization. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Store homogenized sample at -20°C or colder. Perform dilutions in Assay Diluent as necessary.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each Fluorescein sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or Fluorescein standard to the wells of the Fluorescein Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted HRP-Conjugated Anti-Fluorescein Monoclonal Antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer 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. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
6. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
7. Read absorbance of each well on a microplate reader using 450 nm as the primary wavelength.

Example of Results

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

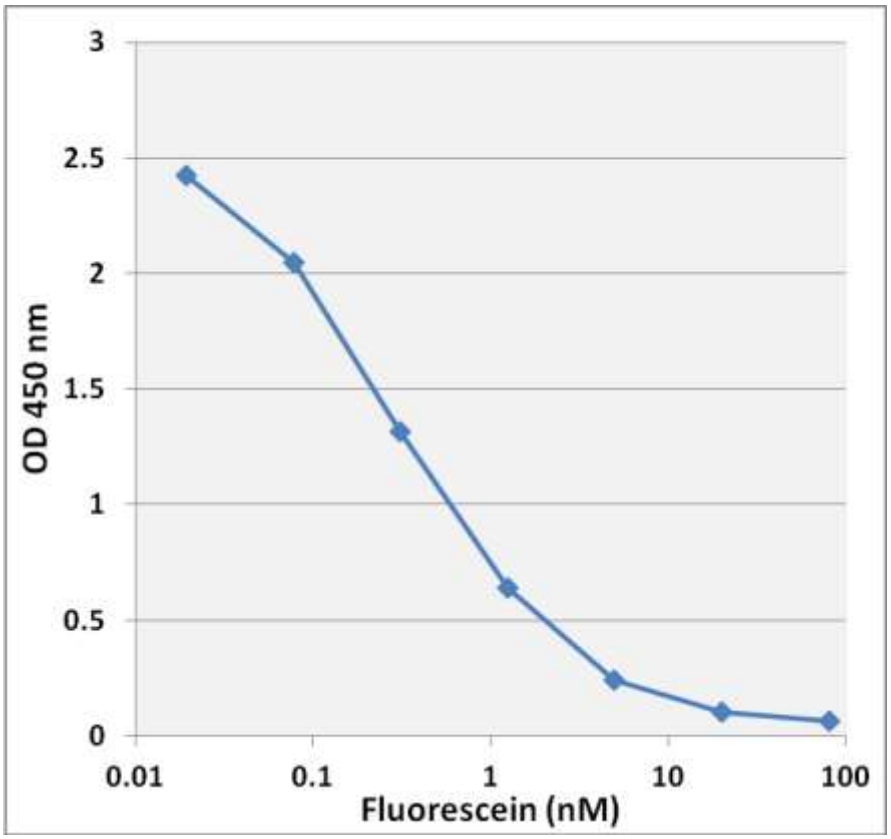

Figure 1: Fluorescein Standard Curve.

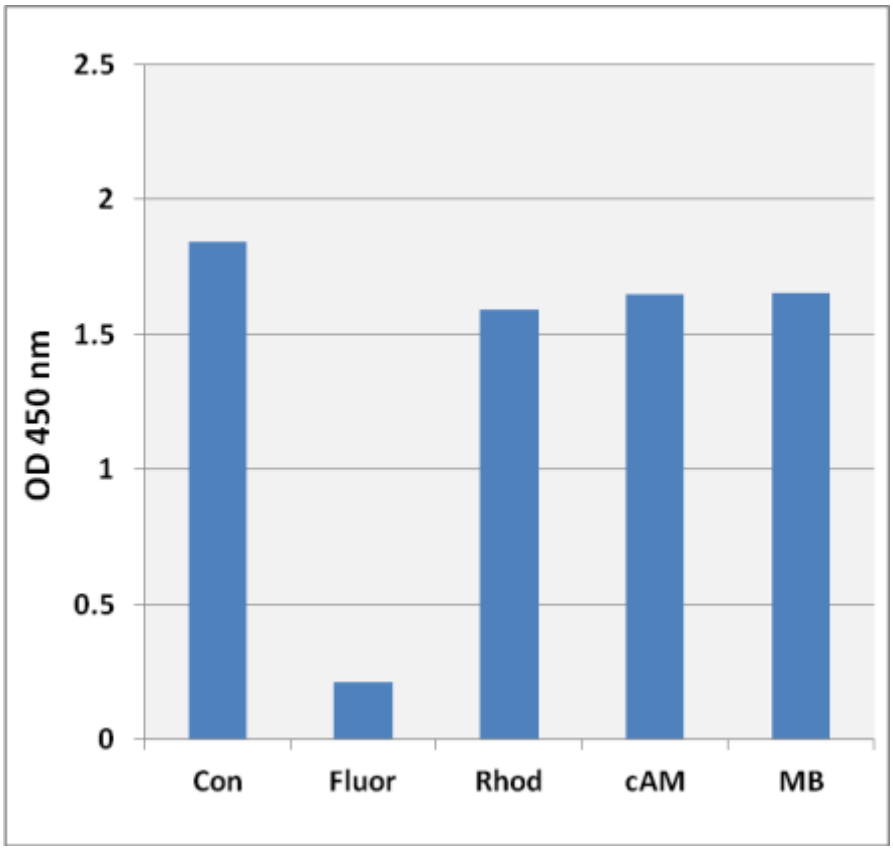

Figure 2: Specificity of Fluorescein ELISA. No drug (Con), 5 nM Fluorescein (Fluor), Rhodamine (Rhod), Calcein AM (cAM) or methylene blue (MB) was measured using the Fluorescein Competitive ELISA Kit.

References

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4. Salih A, Tjoelker MG, Renard J and Pfautsch S (2015). *Plant Physiology*. **167**: 963–971.
5. Duran-Nebreda S and Bassel G (2017). *Bio-Protocol*. **8**: e2791.

Warranty

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com