Product Manual

Rhodamine Competitive ELISA Kit

Catalog Number

AKR-5142 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Rhodamine encompasses a family of structurally related dyes that are derivatives of xanthene. Some important subtypes of the rhodamine family include Rhodamine 123, Rhodamine 6G, and Rhodamine B. They are often used to dye inks and paper. In addition, they are used as a tracer dye in order to find the rate or direction of flow and transport of water. Rhodamine is used as a collective marker in small mammals for studying movements. Rhodamine dyes are fluorescent and can therefore be easily and cheaply detected with a fluorometer. Rhodamines are even used in some lasers. Rhodamine dyes are often used in biological detection applications such as flow cytometry, fluorescence microscopy, ELISA, and fluorescence correlation spectroscopy.

The subtype Rhodamine 123 is used in biochemistry to inhibit the function of mitochondria. Rhodamine 123 inhibits transport processes such as the electron transport chain and slows down inner respiration by binding to the mitochondrial membranes. Rhodamine 123 is also a substrate of P-glycoprotein (usually overexpressed in cancer cells) as well as of multidrug resistance-associated protein (MRP1). There are many rhodamine derivatives used for imaging purposes, such as tetramethylrhodamine (TMR) and its isothiocyanate derivative (TRITC), carboxytetramethylrhodamine (TAMRA), as well as sulforhodamine 101 (and its sulfonyl chloride form Texas Red), and Rhodamine Red.

Cell Biolabs' Rhodamine Competitive ELISA Kit provides a convenient method for the detection of total rhodamine in extracts from cells, tissue, serum, plasma, or foods. The total content of rhodamine in unknown samples is determined by comparison with a rhodamine 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 1 µM rhodamine.

Assay Principle

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

Related Products

1. AKR-5141: Fluorescein Competitive ELISA Kit
2. AKR-120: GFP Quantitation Kit, Fluorometric
3. AKR-121: GFP ELISA Kit
4. AKR-122: RFP ELISA Kit
5. AKR-130: His-Tag Protein ELISA Kit
6. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation 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. Anti-Rhodamine Monoclonal Antibody (Part No. 51421C): One 10 µL vial.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): 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. Rhodamine Standard (Part No. 51422C): One 100 µL vial of 7 mM rhodamine.
2. Rhodamine Conjugate (500X) (Part No. 51423C): One 25 µL vial.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. 1X PBS
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 wavelength)

Storage

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

Preparation of Reagents

- Rhodamine Conjugate Coated Plate:

  Note: The Rhodamine 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 Rhodamine Conjugate by diluting the Rhodamine Conjugate (500X) 1:500 in 1X Conjugate Diluent. Example: Add 10 µL of 500X Rhodamine Conjugate to 4.99 mL of 1X Conjugate Diluent.
3. Add 100 µL of the 1X Rhodamine Conjugate to each well to be tested and incubate overnight at 4ºC. Remove the Rhodamine 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-Rhodamine Antibody and Secondary Antibody: Immediately before use, dilute the Anti-Rhodamine 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 Rhodamine standards in the concentration range of 0 to 70 µM by diluting the Rhodamine Standard in Assay Diluent (Table 1).

Standard Tubes	7 mM Rhodamine Standard (µL)	Assay Diluent (µL)	Rhodamine (µM)
1	10	990	70
2	500 of Tube #1	500	35
3	500 of Tube #2	500	17.5
4	500 of Tube #3	500	8.75
5	500 of Tube #4	500	4.38
6	500 of Tube #5	500	2.19
7	500 of Tube #6	500	1.09
8	0	500	0

Table 1. Preparation of Rhodamine 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 Rhodamine sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or Rhodamine standard to the wells of the Rhodamine Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted Anti-Rhodamine 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. Add 100 µL of the diluted Secondary Antibody-HRP Enzyme Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.
8. 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.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
10. Read absorbance of each well on a microplate reader using 450 nm as the primary wavelength.

Example of Results

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

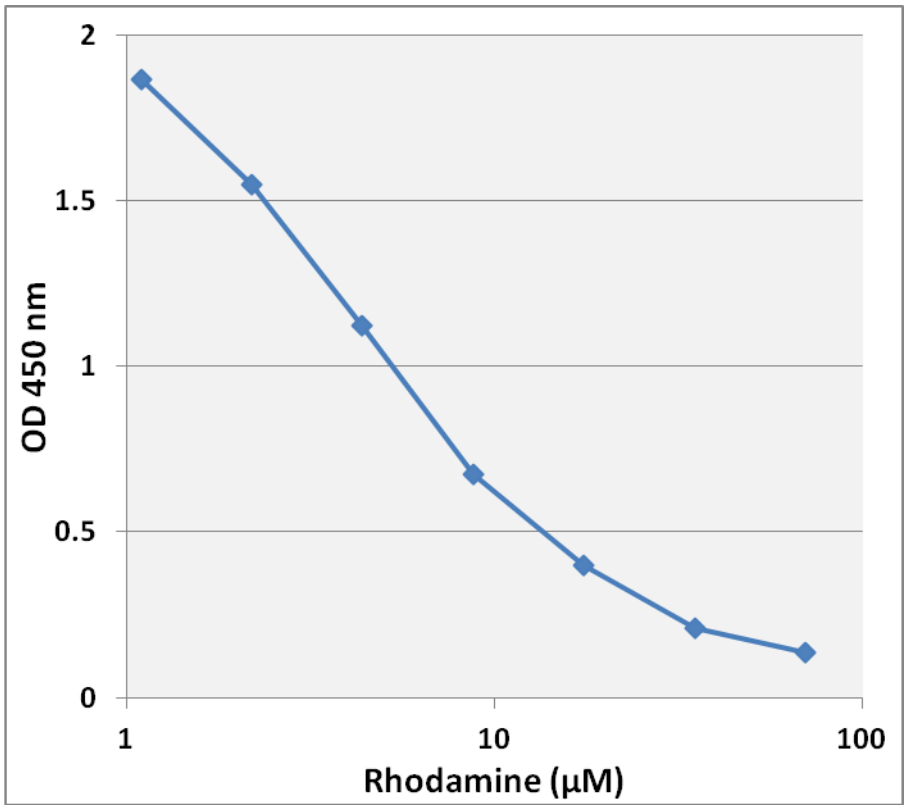

Figure 1: Rhodamine Standard Curve.

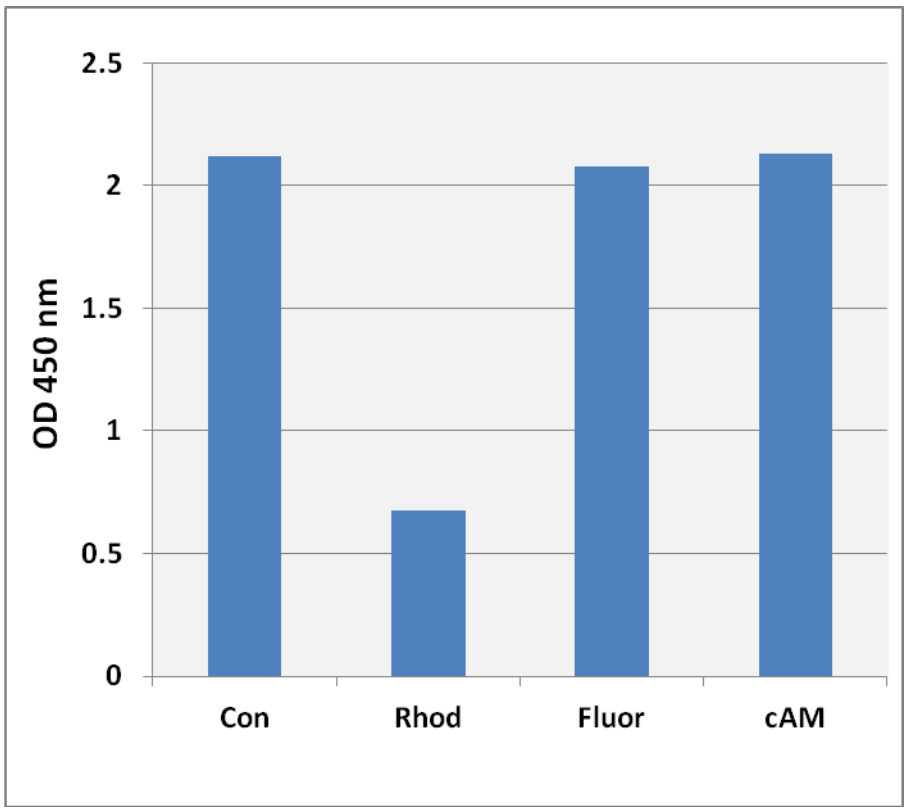

Figure 2: Specificity of Rhodamine ELISA. No drug (Con), 8.75 µM Rhodamine (Rhod), Fluorescein (Fluor), or Calcein AM (cAM) was measured using the Rhodamine Competitive ELISA Kit.

References

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3. Forster S, Thumser AE, Hood SR, and Plant N. (2012) *PLOS One* **7**:1-9.
4. Papillion Y, Buffiere L, and Butet A. (2002) *Acta Theriologica*. **47**:491-497.
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Warranty

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