RayBio® FAD Colorimetric/Fluorometric Assay Kit

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RayBio® FAD Colorimetric/Fluorometric Assay Kit Protocol

(Cat#: 68-FAD-S100)

RayBiotech, Inc.

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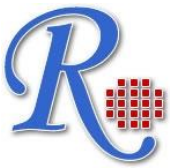

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I. INTRODUCTION

Flavin Adenine Dinucleotide (FAD) is a redox cofactor which plays an important role in metabolism. FAD exists in different redox states and cycles between FAD, FADH and $FADH_2$. The primary sources of reduced FAD in eukaryotic metabolism are the citric acid cycle and the beta oxidation reaction pathways. In BioVision's FAD Assay Kit, FAD functions as the cofactor of an oxidase which catalyzes the formation of a product generating color and fluorescence. FAD can be detected by either colorimetric (OD = 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The kit provides a rapid, simple, ultra-sensitive, and reliable test suitable for high throughput assay of FAD. The lower limit of detection is less than 1 nM FAD.

II. REAGENTS

Components	68-FAD-S100	Cap Code	Part Number
FAD Assay Buffer	25 ml	WM	Item A
OxiRed Probe (in DMSO)	200 µl	Red	Item B
FAD Enzyme Mix (lyophilized)	1 Vial	Green	Item C
FAD Standard (1nmol, lyophilized)	1 Vial	Yellow	Item D
Stop Solution	1.2 ml	Blue	Item E

III. STORAGE AND HANDLING

Store the kit at -20°C and protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Please read the entire protocol before performing the assay. **Avoid repeated freeze/thaw cycles as they will inactivate the components.**

IV. REAGENT PREPARATION

Probe: Ready to use as supplied. Warm to room temperature to allow DMSO solution to thaw before use. Store at -20°C, protect from light. Use within two months.
Enzyme Mix: Dissolve in 220 µl Distilled Water. Store at -20°C. Use within two months.
FAD Standard: Dissolve in 100 µl DMSO to generate a 10pmol/µl (10 µM) FAD standard solution.

V. Assay Procedure:

1. **Standard Curve Preparation:**
   For Colorimetric Assays: Dilute the 10 pmol/µl FAD Standard solution to 0.2 pmol/µl by adding 10 µl of FAD Standard to 490 µl of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay

Buffer to generate 0, 0.4, 0.8, 1.2, 1.6, 2 pmol/well of the FAD Standard. Use freshly diluted Standard each time you perform the assay.
For Fluorometric Assays: Dilute the FAD Standard solution to 0.02 pmol/µl by adding 10 µl of the 10 pmol/µl FAD Standard to 490 µl of Assay Buffer, mix well. Then take 10 µl to 90 µl of Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.04, 0.08, 0.12, 0.16, 0.2 pmol/well of the FAD Standard.

2. **Sample Preparation:**
Considerations: Serum samples should be collected using EDTA to inhibit enzymes that may degrade FAD. Approximate sample amount per assay: ~0.1 - 0.5 mg tissue; ~10,000 - 100,000 cultured cells, ~0.1 - 20 µl serum. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the standard curve range
Homogenize tissue (5 - 20 mg) or cells (1 x 10^6) in 400 µl of Assay Buffer and centrifuge to remove insoluble material at 13,000 x g for 3 mins. Deproteinize sample using a perchloric acid precipitation technique to release FAD from proteins. After deproteinization, add samples directly to a 96 well plate, and then bring the volume to 50 µl/well with Assay Buffer.

3. **Reaction Mix:** Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Sample
Assay Buffer	46 µl
OxiRed Probe	2 µl*
Enzyme Mix	2 µl

***Note:** Fluorometric measurements are 10x more sensitive than colorimetric measurements. Using less OxiRed Probe (1uL or less) in the reaction mix significantly reduces assay background
- Add 50 µl of the Reaction Mix into each well.

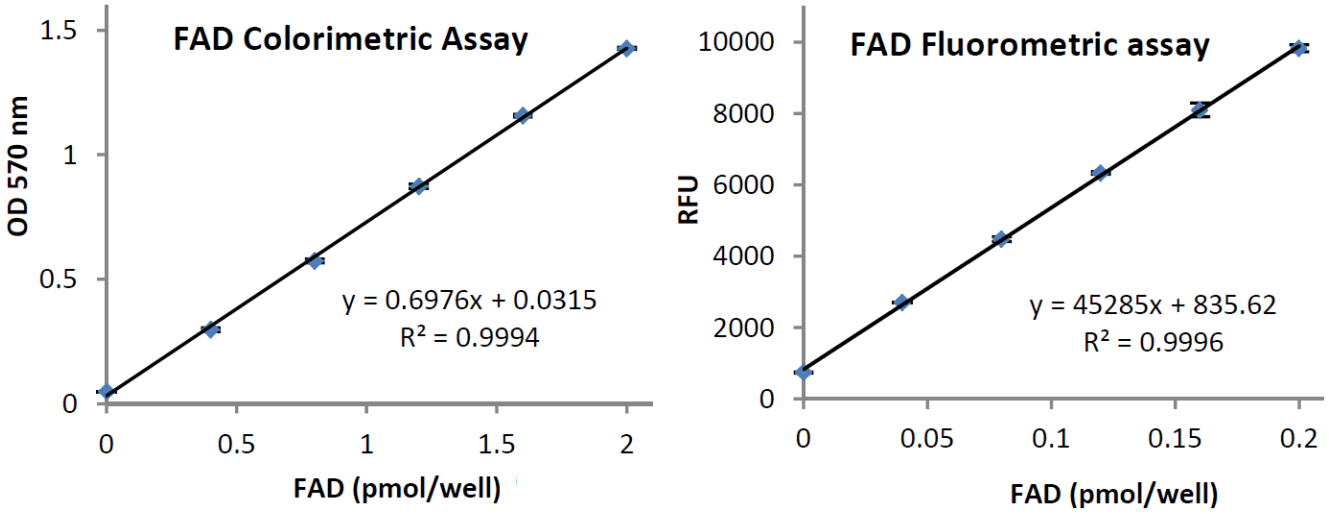
4. **Incubation:** Incubate at Room Temperature.
5. **Measuring the Assay:** Measure optical density (OD) at 570 nm for the colorimetric assay or Ex/Em = 535/587 nm for the Fluorometric assay in a micro plate reader. Read the samples and standards every 5 min until readings are in range for the standard curve points, but not saturating. You can stop the reactions by adding 10 µl of Stop Solution. Gently shake the plate to mix. The

reaction is stable for 24 hr after adding stop solution. Use the data on the time that shows maximum linear readings. Generally, the reaction is linear with time until OD 570 nm reaches 1.8 in the colorimetric assays.

6. **Calculation:** Correct background by subtracting the value derived from the 0 FAD control sample from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings). Plot the FAD standard Curve, and the FAD concentrations of the test samples can then be calculated based on the following equation:

$$C = S_a / S_v * D \quad \text{pmol/µl or µM}$$

Where: **S_a** is the sample amount (in pmol) from standard curve.
S_v is the sample volume (µl) added into the reaction wells.
FAD has a molecular weight of 785.55 g/mol.



FAD Standard Curves for both Colorimetric (left) and Fluorometric (Right): Assay was performed as described, and measurement determined after 15min of incubation

This product is for research use only.