



Flavonoid Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1234

(Version 1.4A)

Detection and Quantification of Flavonoid Content in Tissue
extracts and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signalling pathways and antioxidant effects. These molecules are found in a variety of fruits and vegetables. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water. They consist of two benzene rings connected by a short three carbon chain. One of the carbons in this chain is connected to a carbon in one of the benzene rings, either through an oxygen bridge or directly, which gives a third middle ring. The flavonoids can be divided into six major subtypes, which include chalcones, flavones, isoflavonoids, flavanones, anthoxanthins and anthocyanins.

Flavonoid Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of Flavonoid in a variety of samples. The Flavonoid is subsequently measured by a coupled chemical reaction system with a colorimetric readout at 420 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dye ReagentA	1 ml x 1	4 °C
Dye ReagentB	1 ml x 1	4 °C
Dye Reagent C	8 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 420 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer to generate 10 mmol/L of standard stock solution, store at 4 °C for 2-4 weeks or -20°C for 6 months after reconstitution. Then dilute to 5 mmol/L standard top solution by adding 0.5 ml stock solution into 0.5 ml Assay Buffer. Perform 2-fold serial dilutions of the top standard solution using Assay Buffer to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.313/0.156/0.078 mmol/L.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer, then transfer it to the microcentrifuge tubes; incubate at boiling water bath for 30 mins; centrifuged at 10,000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	90 μ l	90 μ l	90 μ l
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Assay Buffer	--	--	10 μ l
Dye Reagent A	10 μ l	10 μ l	10 μ l
Mix, incubate at room temperature for 5 minutes.			
Dye Reagent B	10 μ l	10 μ l	10 μ l
Mix, incubate at room temperature for 5 minutes.			
Dye Reagent C	80 μ l	80 μ l	80 μ l
Keep it at room temperature for 10 minutes, record absorbance measured at 420 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.2 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

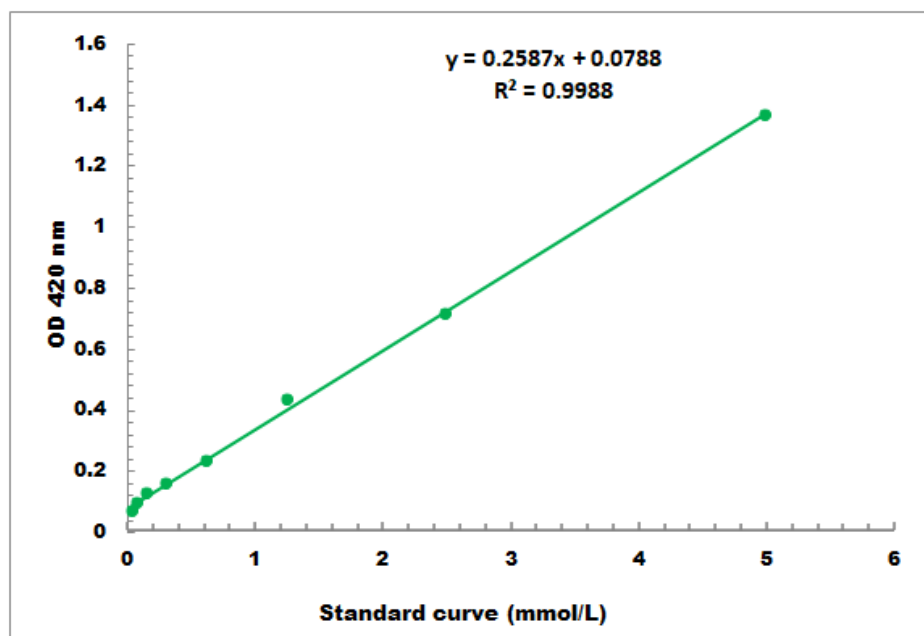
V_{Sample} : the volume of sample in assay procedure, μl

V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L