

## **Flavonoid Microplate Assay Kit**

**Cat #: orb707391 (manual)**

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

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

## 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 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.

**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 Reagent A	1 ml x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Dye Reagent C	8 ml x 1	4 °C
Standard	Powder x 1	4 °C
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**Note:**

**Standard:** add 1 ml Reaction Buffer to dissolve before use; then add 0.5 ml into 0.5 ml Reaction Buffer, mix, the concentration will be 5 mmol/L.

**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

## 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.

## ASSAY PROCEDURE

Add following reagents into the microplate:

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

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) 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.

## CALCULATION

### 1. According to the weight of sample

$$\begin{aligned}\text{Flavonoid (mmol/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (W \times V_{\text{Sample}} / \\ &\quad V_{\text{Assay}}) \\ &= 0.005 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W\end{aligned}$$

### 2. According to the volume of sample

$$\begin{aligned}\text{Flavonoid (mmol/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / V_{\text{Sample}} \\ &= 0.005 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}})\end{aligned}$$

$C_{\text{Standard}}$ : the concentration of standard, 5 mmol/L = 0.005 mmol/ml;

W: the weight of sample, g;

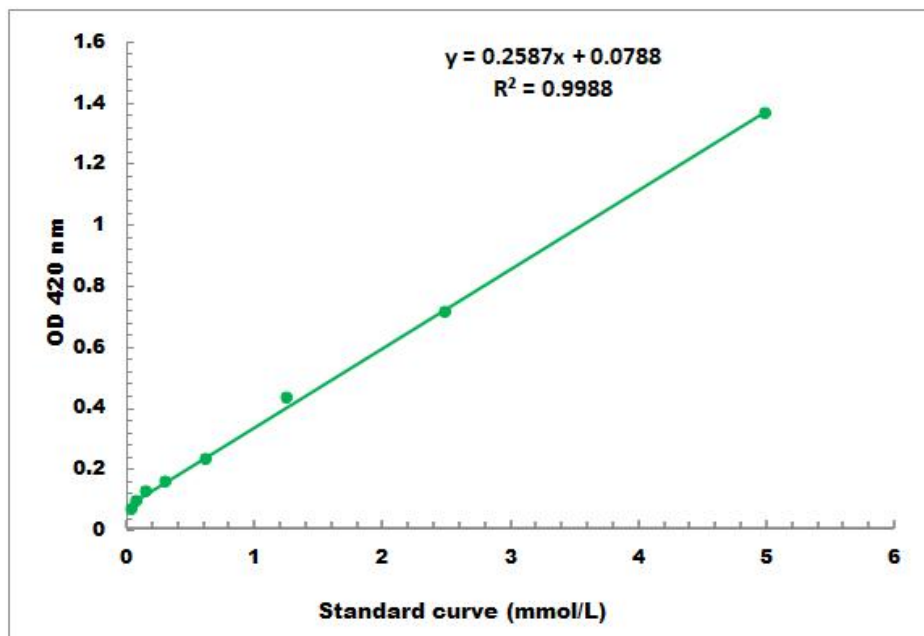
$V_{\text{Standard}}$ : the volume of standard, 0.01 ml;

$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay Buffer, 1 ml.

## 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