Flavonoid Microplate Assay Kit

User Manual

Catalog # CAK1234

(Version 1.2A)

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

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

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 benzine 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.

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 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.

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. 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.

V. 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\text{Flavonoid (mmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 0.005 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

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

$$= 0.005 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

W: the weight of sample, g;

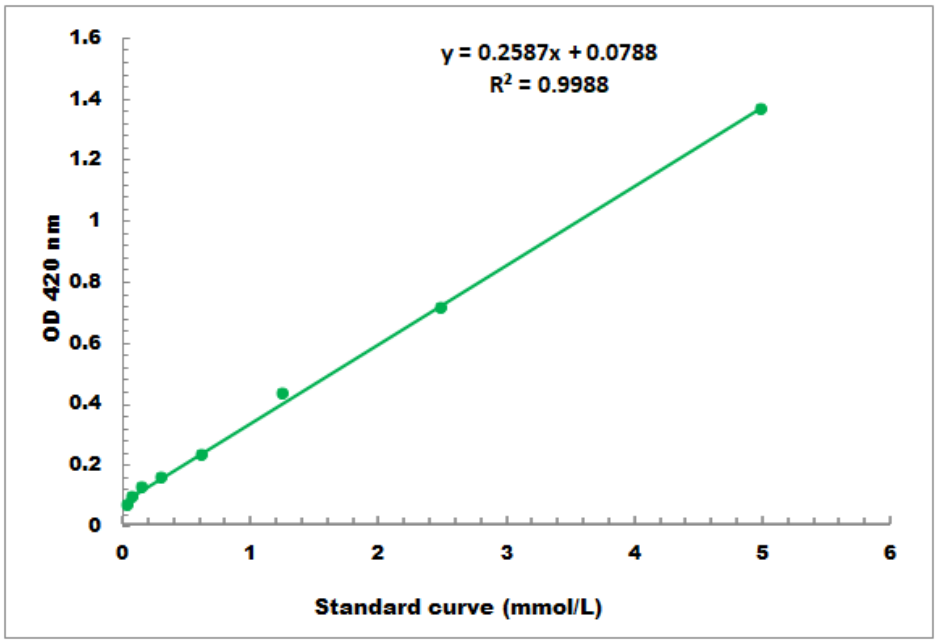
$V_{Standard}$: the volume of standard, 0.01 ml;

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

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

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES