



FlavoneMicroplate Assay Kit

User Manual

Catalog # CAK1233

(Version 1.2A)

Detection and Quantification of Flavone 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

Flavones are molecules present in most plants that are an important component of some human diets. Epidemiological evidence shows the beneficial effects of these molecules in cardiovascular and neuropathological diseases. Experimental evidence in vitro and in vivo has confirmed the neuroprotective effects in neurons in culture against oxidative insults and in models of focal ischemia and experimental parkinsonism.

Flavone Microplate Assay Kit provides a convenient tool for sensitive detection of Flavone in a variety of samples. The Flavone 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	15 ml x 1	4 °C
Dye ReagentA	5 ml x 1	4 °C
Dye ReagentB	3 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 0.2 ml into 0.8 ml Reaction Buffer, mix, the concentration will be 2 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; centrifuge 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	20 μ l	--	--
Standard	--	20 μ l	--
Assay Buffer	--	--	20 μ l
Reaction Buffer	100 μ l	100 μ l	100 μ l
Dye Reagent A	50 μ l	50 μ l	50 μ l
Dye Reagent B	30 μ l	30 μ l	30 μ l
Keep it at room temperature for 30 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

$$\begin{aligned}\text{Flavone (mmol/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \\ &\quad \times V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.002 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

2. According to the volume of sample

$$\begin{aligned}\text{Flavone (mmol/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} \\ &= 0.002 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

C_{Standard} : the concentration of Standard, 2 mmol/L = 0.002 mmol/ml;

W: the weight of sample, g;

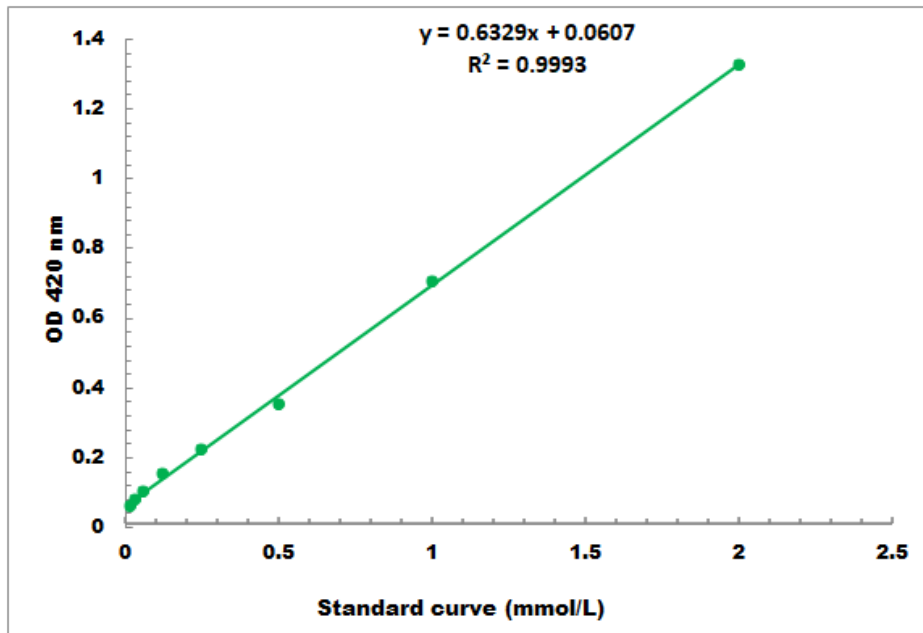
V_{Standard} : the volume of standard, 0.02 ml;

V_{Sample} : the volume of sample, 0.02 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: 0.02 mmol/L - 2 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