Flavone

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1233

(Version 1.3A)

Detection and Quantification ofFlavoneContentin Tissue extractsand Other biological fluidsSamples.

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

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.

FlavoneColorimetric 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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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.Dissolvein1 mlReaction Bufferto generate 10 mmol/L of standardstock solution, store at 4 °C for 2-4 weeks or -20°C for 6 monthsafter reconstitution.Then dilute to2 mmol/Lstandard top solutionby adding0.2 ml stock solution into 0.8 ml Reaction Buffer.Perform 2-fold serial dilutions of the top standardsolution using Reaction Bufferto make the standard curve.The concentration of standard curve could be2/1/0.5/0.25/0.125/0.0625/0.0312mmol/L.

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

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer, then transfer it to the microcentrifuge tubes; incubate at boiling water bath for30 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 intothe microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	100 µl	100 µl	100 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Assay Buffer	--	--	20 µl
Dye Reagent A	50 µl	50 µl	50 µl
Dye Reagent B	30 µl	30 µl	30 µl
Keep it at room temperature for 30minutes, 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. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

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

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

2.2According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

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

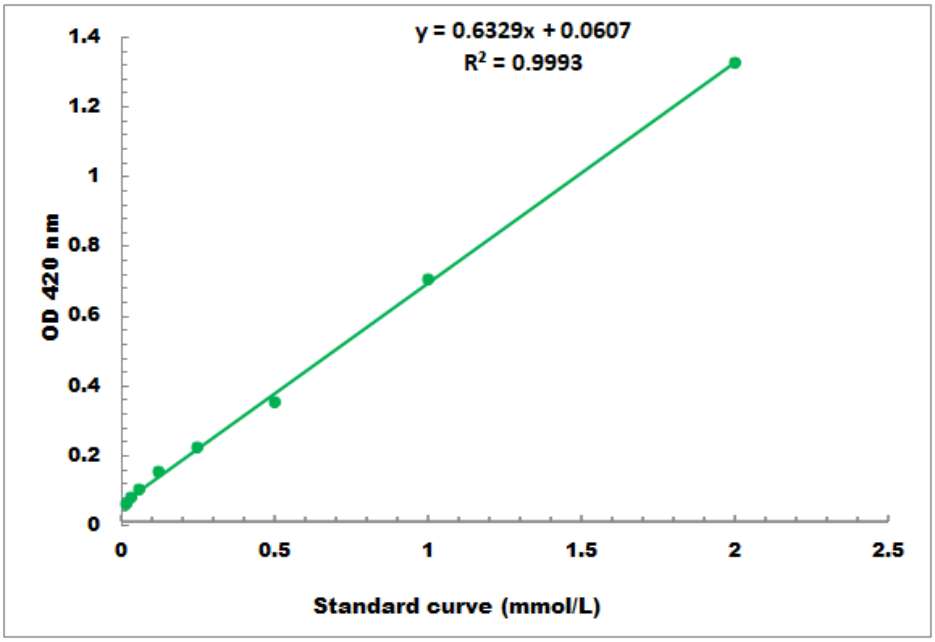
V_{Sample}: the volume of sample in assay procedure, μl

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: 0.02 mmol/L - 2 mmol/L