Flavonoid Assay Kit

ARG83418 Flavonoid Assay Kit can be used to measure Flavonoid in tissue extracts and other biological fluids.

Catalog number: ARG83418

Package: 96 wells

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INTRODUCTION

Flavonoids are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in the diets of humans.Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings and a heterocyclic ring.

PRINCIPLE OF THE ASSAY

The Flavonoid Assay Kit can measure Flavonoid in tissue extracts and other biological fluids. The increase in absorbance at 420 nm is directly proportional to reactants of the reaction between substrate and Flavonoid.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Reaction Buffer	10 ml (ready to use)	4°C
Reagent Dye A	1 ml (ready to use)	4°C
Reagent Dye B	1 ml (ready to use)	4°C
Reagent Dye C	8 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 420 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all components store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue - 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.

*Note: liquid samples can detect directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to dissolve standard, then add 0.5 ml into 0.5 ml Reaction Buffer. The concentration will be 5 µmol /ml. Perform 2-fold serial dilution of the top standards to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl Sample** into Sample wells.
2. Standard wells: Add **10 µl Standard** into Standard wells.
3. Add **90 µl Reaction Buffer** to each wells.
4. Add **10 µl Reagent Dye A** to each wells.
5. Mix well. Incubate at **RT** for **5 min**
6. Add **10 µl Reagent Dye B** to each wells.
7. Mix well. Incubate at **RT** for **5 min**
8. Add **80 µl Reagent Dye C** to each wells.
9. Mix well. Incubate at **RT** for **10 min**
10. Read the OD at **420nm**

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Assay Buffer	-		10 µl
Reagent Buffer	90 µl	90 µl	90 µl
Reagent Dye A	10 µl	10 µl	10 µl
Mix well. Incubate at RT for 5 min			
Reagent Dye B	10 µl	10 µl	10 µl
Mix well. Incubate at RT for 5 min			
Reagent Dye C	80 µl	80 µl	80 µl
Mix well. Incubate at RT for 10 min			
Read the OD at 420nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 ml;

$V_{standard}$: the volume of standard, 10 µl = 0.01 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml.

B. Formula:

a). According to the weight of sample

Flavonoid (mmol/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{assay})]$

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

b). According to the volume of sample

Flavonoid (mmol/ml) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample})]$

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

3. Detection range:

The detection range is from 0.05 µmol/ml- 5 µmol/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.