Proanthocyanidin Assay Kit

ARG83419 Proanthocyanidin Assay Kit can be used to measure Proanthocyanidin in tissue extracts and other biological fluids.

Catalog number: ARG83419

Package: 96 wells

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INTRODUCTION

Proanthocyanidins are a class of polyphenols found in many plants, such as cranberry, blueberry, and grape seeds. Chemically, they are oligomeric flavonoids. Many are oligomers of catechin and epicatechin and their gallic acid esters. More complex polyphenols, having the same polymeric building block, form the group of tannins.

PRINCIPLE OF THE ASSAY

The Proanthocyanidin Assay Kit can measure Proanthocyanidin in tissue extracts and other biological fluids. The increase in absorbance at 500 nm is directly proportional to reactants of the reaction between substrate and Proanthocyanidin.

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	8 ml (ready to use)	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Reagent Dye Diluent	10 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 500 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 60 °C water bath for 1 hour; 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 **Assay Buffer** to dissolve standard, the concentration will be 10 µmol /ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Reagent Dye**: Add **10 ml** of **Reagent Dye Diluent** to dissolve Reagent Dye before use

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **20 µl Sample** into Sample wells.
2. Standard wells: Add **20 µl Standard** into Standard wells.
3. Add **80 µl Reaction Buffer** to each wells.
4. Add **100 µl Reagent Dye** to each wells.
5. Mix well. Incubate at **37°C** for **20 min**
6. Read the OD at **500nm**

Reagent	Sample	Standard	Blank
Sample	20 µl	-	-
Standard	-	20 µl	-
Assay Buffer	-		20 µl
Reagent Buffer	80 µl	80 µl	80 µl
Reagent Dye	100 µl	100 µl	100 µl
Mix well. Incubate at **37°C** for **20 min**			
Read the OD at **500nm**			

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, 0.01 mmol /ml=10 μmol /ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 20 μl = 0.02 ml;

$V_{standard}$: the volume of standard, 20 μl = 0.02 ml;

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

B. Formula:

a). According to the weight of sample

Proanthocyanidin (mmol/g) =

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

$=0.01 \times (OD_{Sample} - OD_{blank}) / [(OD_{Standard} - OD_{Blank}) \times W]$

b). According to the volume of sample

Proanthocyanidin (mmol/ml) =

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

$=0.01 \times (OD_{Sample} - OD_{blank}) / (OD_{Standard} - OD_{Blank})$

3. Detection range:

The detection range is from 0.1 µmol/ml - 10 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.