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Saponin Microplate Assay Kit

Cat#: orb759224 (User Manual)

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

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I. INTRODUCTION

Saponin, any of numerous substances, occurring in plants, that form stable foams with water, including the constituents of digitalis and squill that affect the heart and another group that does not affect the heart.

Saponins affecting the heart have been used as arrow and spear poisons by African and South American natives. Digitalis, from purple foxglove, Digitalis purpurea, was introduced into heart therapy in 1785 by the Scottish physician William Withering. The non-cardiac-active saponins include digitonin, which was recognized in digitalis preparations in 1875; and dioscin, the precursor of diosgenin, which is obtained from a Mexican yam.

Saponin Microplate Assay Kit provides a convenient tool for sensitive detection of Saponin in a variety of samples. The Saponin is subsequently measured by a coupled chemical reaction system with a colorimetric readout at 560 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	RT
Dye Reagent A	Powder x 1	4 °C
Dye Reagent A Diluent	1.8 ml x 1	4 °C
Dye Reagent B	4 ml x 1	4 °C

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Stop Solution	15 ml x 1	4 °C
Standard	Powder x 1	4 °C
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Note:
Standard: add 1 ml Assay Buffer to dissolve before use; then add 0.5 ml into 0.5 ml Assay Buffer, mix, the concentration will be 5 mmol/L.

Dye Reagent A: add 1.8 ml Dye Reagent A Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 560 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 50 °C water bath for 1 hour; 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
Dye Reagent A	15 µl	15 µl	15 µl
Dye Reagent B	40 µl	40 µl	40 µl
Mix, incubate at 50 °C for 20 minutes.			
Stop Solution	135 µl	135 µl	135 µl
Mix, record absorbance measured at 560 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.

VI. CALCULATION

1. According to the weight of sample

$$\text{Saponin (mmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$
$$= 0.005 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

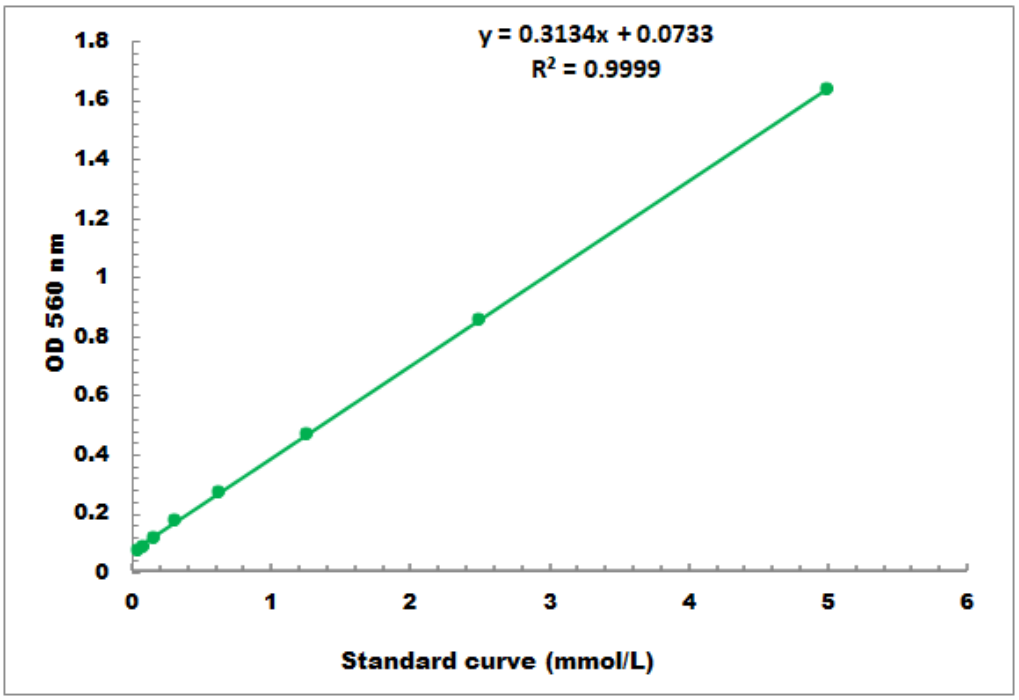
$$\text{Saponin (mmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$
$$= 0.005 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: 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