Saponin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1237

(Version 1.3C)

Detection and Quantification ofSaponinContentin Tissue extractsand Other biological fluidsSamples.

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

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.

SaponinColorimetric 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 540 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Dye Reagent Diluent	5 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1.Sulfuric acid(98%)- Analytical Reagent Grade

2. Microplate reader to read absorbance at 540 nm

3. Pipettor, multi-channel pipettor

4. Pipette tips

5. Mortar

6. Ice-water bath

7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer to generate stock standard solution; then add 0.1 ml stock solution into 0.1 ml Assay Bufferto generate 5 mmol/L of top standardsolution.Perform 2-fold serial dilutions of the top standardsolution using Assay Bufferto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.63/0.31/ 0.16/0.08/0.04 mmol/L.

Dye Reagent: Add 5 ml Dye Reagent Diluent to dissolve before use. Store at 4 °Cin dark for 1 weekafter reconstitution.

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 50 °C water bath for1 hour; 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

Place the 96-Well Microplate in an ice-water bath.Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Assay Buffer	--	--	50 µl
Dye Reagent	50µl	50µl	50µl
Add 100 µl sulfuric acid down the inner wallslowly, mix thoroughly while keeping in the ice bath.			
Incubate at 60 °C for 15 minutes. Record absorbance measured at 540 nm after cooling.			

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

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

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1According 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

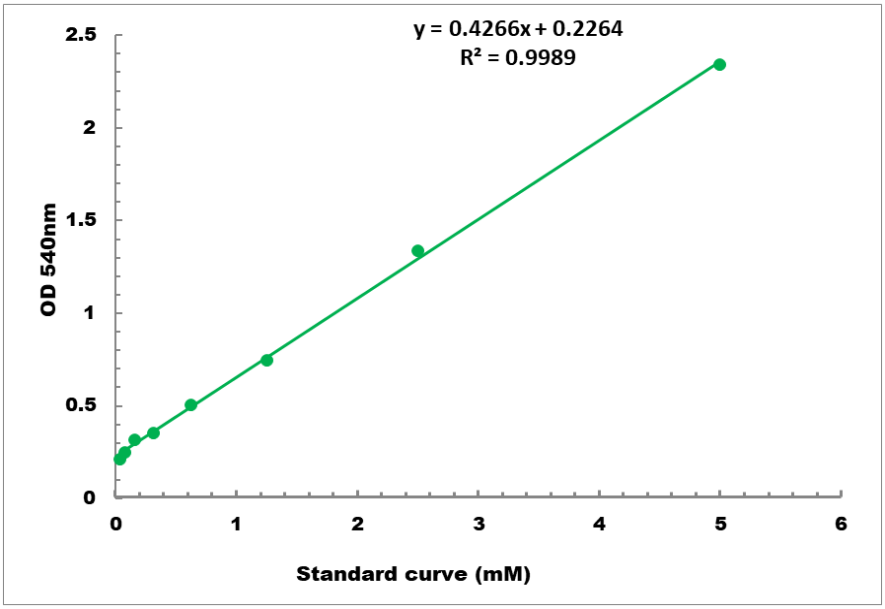
V_{Sample}: the volume of sample in assay procedure, μl

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: 0.04 mmol/L - 5 mmol/L