Saponin Colorimetric Microplate Assay Kit

Cat #: orb759224 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

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

INTRODUCTION

Saponins are a diverse group of naturally occurring plant compounds that form stable foams when mixed with water. They include cardiac-active compounds found in digitalis and squill, as well as non-cardiac-active compounds. Cardiac-active saponins have historically been used as arrow and spear poisons by indigenous peoples in Africa and South America. Digitalis, derived from purple foxglove (Digitalis purpurea), was introduced into cardiac therapy in 1785 by Scottish physician William Withering. Non-cardiac-active saponins include digitonin, first identified in digitalis preparations in 1875, and dioscin, a precursor of diosgenin obtained from Mexican yam. The Saponin Colorimetric Microplate Assay Kit provides a convenient and sensitive method for detecting saponins in a variety of samples. Saponin levels are measured using a coupled chemical reaction system with a colorimetric readout at 540 nm.

KIT COMPONENTS

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

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

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 Buffer to generate 5 mmol/L of top standard solution. Perform 2-fold serial dilutions of the top standard solution using Assay Buffer to 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 °C in dark for 1 week after reconstitution.

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.

ASSAY PROCEDURE

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

Reagent*	Sample**	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Assay Buffer	--	--	50 µl
Dye Reagent	50 µl	50 µl	50 µl
Add 100 µl sulfuric acid down the inner wall slowly, 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.

CALCULATION

1. Calculate the 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

2.1 According to the weight of sample

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

2.2 According 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

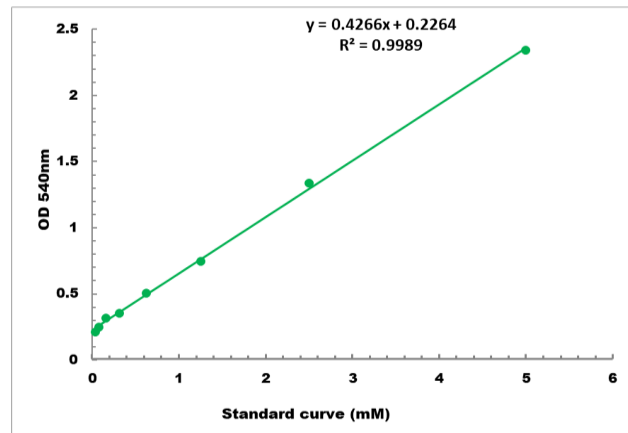
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558