Saponin Assay Kit

ARG83426 Saponin Assay Kit can be used to measure Saponin in tissue extracts and other biological fluids.

Catalog number: ARG83426

Package: 96 wells

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INTRODUCTION

Saponins, also selectively referred to as triterpene glycosides, are bitter-tasting usually toxic plant-derived organic chemicals that have a foamy quality when agitated in water. Saponins are widely distributed but found particularly in soapwort, a flowering plant, the soapbark tree and soybeans.

PRINCIPLE OF THE ASSAY

The Saponin Assay Kit can measure Saponin in tissue extracts and other biological fluids. The increase in absorbance at 540 nm is directly proportional to reactants of the reaction between substrate and Saponin.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all components store at 4°C.

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
Reagent Dye	1 vial (lyophilized)	4°C, keep in dark
Reagent Dye Diluent	5 ml	4°C
Plate Adhesive Strips	3 Strips	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Sulfuric acid (98%)- Analytical Reagent Grade
- Ice-water bath

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 50 °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, then add 0.1 ml into 0.1 ml **Assay Buffer**, the concentration will be 5 µmol /ml. Perform 2-fold serial dilution of the top standards to make the standard curve. The concentration of standard curve could be **5 µmol /ml, 2.5 µmol /ml, 1.25 µmol /ml, 0.63 µmol /ml, 0.31 µmol /ml, 0.16 µmol /ml, 0.08 µmol /ml, 0.04 µmol /ml.**
- **Reagent Dye** : Add **5ml** of **Reagent Dye Diluent** to dissolve Reagent Dye before use. Store at 4 °C in dark for 1 week after reconstitution.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **50 µl** of **Sample** into Sample wells.
2. Standard wells: Add **50 µl** of **Standard** into Standard wells.
3. Blank wells: Add **50 µl Assay Buffer** into Blank wells
4. Add **50 µl** of **Reagent Dye** to each wells
5. Add **100 µl Sulfuric acid** to down the inner wall slowly
6. Mix thoroughly while keeping in the ice bath.
7. Incubate at **60°C** for **15 min**.
8. Read the OD at **540 nm**.

Summary of Saponin Assay Kit Procedure

Reagent	Sample	Standard	Blank
Sample	50 µl	-	-
Standard	-	50 µl	-
Assay Buffer	-	-	50 µl
Reagent Dye	50 µl	50 µl	50 µl
Sulfuric acid	100 µl	100 µl	100 µl
Mix thoroughly while keeping in the ice bath			
Incubate at 60 °C for 15 minutes.			
Read the OD at 540 nm			

Note:

1. Reagents must be added sequentially and should not be premixed prior to addition.
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.

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, 5 µmol /ml = 0.005 mmol /ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 50 µl = 0.05 ml;

$V_{standard}$: the volume of standard, 50 µl = 0.05 ml;

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

B. Formula:

a). According to the weight of sample

Saponin (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

Saponin (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 0.04 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 serially diluted standards are necessary for this kit.