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 560 nm is directly proportional to reactants of the reaction between substrate and Saponin.

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)	RT
Reagent Dye A	1 vial (lyophilized)	4°C
Reagent Dye A Diluent	1.8 ml	4°C
Reagent Dye B	4 ml (ready to use)	4°C
Stop Solution	15 ml (ready to use)	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 560 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 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 0.5 ml into 0.5 ml **Assay Buffer**, the concentration will be 5 µmol /ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Reagent Dye A**: Add **1.8 ml** of **Reagent Dye A Diluent** to dissolve Reagent Dye before use

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl Sample** into Sample wells.
2. Standard wells: Add **10 µl Standard** into Standard wells.
3. Add **15 µl Reagent Dye A** to each wells.
4. Add **40 µl Reagent Dye B** to each wells.
5. Mix well. Incubate at **50°C** for **20 min**.
6. Add **135 µl Stop Solution** to each wells.
7. Read the OD at **560nm**.

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Assay Buffer	-	-	10 µl
Reagent Dye A	15 µl	15 µl	15 µl
Reagent Dye B	40 µl	40 µl	40 µl
Mix well. Incubate at **50°C** for **20 min**			
Stop Solution	135 µl	135 µl	135 µl
Read the OD at **560nm**			

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, 0.005 mmol /ml=5 μmol /ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 μl = 0.01 ml;

$V_{standard}$: the volume of standard, 10 μl = 0.01 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 0.05 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 serial diluted standards are not necessary for this kit.