Tannase Assay Kit

ARG83427 Tannase Assay Kit can be used to measure Tannase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83427

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Tannins is a key enzyme in the degradation of gallotannins and ellagicitannins, two types of hydrolysable tannins.Tannase belongs to the family of hydrolases, specifically those acting on carboxylic ester bonds.

PRINCIPLE OF THE ASSAY

Tannase Assay Kit determined Tannase activity in various samples. Tannase Assay Kit is based on hydrolysis of substrate to gallic acid. The tannase activity can be measured at a colorimetric readout at 520 nm

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Positive Control	1 vial (lyophilized)	-20°C
Assay Buffer	4 X 30 ml	4°C
Reaction Buffer	4 ml	4°C
Substrate	1 vial (lyophilized)	4°C
Substrate Diluent	4 ml	4°C
Reagent Dye A	1 vial (lyophilized)	4°C
Reagent Dye A Diluent	6 ml	4°C
Reagent Dye B	4 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 520 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 Positive Control at -20°C, all other component 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.

For cell and bacteria - Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×106 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

For tissue - Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Reconstitute the Standard with **1 ml** of **Distilled water**. Allow the Standard keep on bench for few minutes make sure the Standard is dissolved completely. Then add **100 µl Standard** into **900 µl distilled water**. The concentration will be 2 µmol/ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Substrate:** Reconstitute the Substrate with **4 ml** of **Substrate Diluent**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Dye Reaction A:** Add **6 ml** of **Reagent Dye A Diluent** before use. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Positive Control:** Add **0.1 ml** of **Distilled water** before use. Allow the Positive Control keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **40 µl Substrate** into each tubes.
2. Add **40 µl Reaction Buffer** into each tubes.
3. Add **20 µl Sample** into Sample tubes.
4. Add **20 µl Standard** into Standard tubes.
5. Add **20 µl Positive Control** into Positive Control.
6. Mix well. Incubate at **30°C** for **10 min**.
7. Add **60 µl Reagent Dye A** into all wells.
8. Add **40 µl Reagent Dye B** into all wells.
9. Mix well. Incubate at **RT** for **10 min**. Read the OD at **520 nm**.

Summary of Tannase Assay Procedure

Reagent	Sample	Control	Standard	Blank
Substrate	40 µl	40 µl	40 µl	40 µl
Reaction Buffer	40 µl	40 µl	40 µl	40 µl
Sample	20 µl	-	-	-
Positive Control	-	20 µl	-	-
Standard	-	-	20 µl	-
Distilled water	-	-	-	20 µl
Mix well. Incubate at 30°C for 30 min				
Reagent Dye A	60 µl	60 µl	60 µl	60 µl
Reagent Dye B	40 µl	40 µl	40 µl	40 µl
Mix well. Incubate at RT for 10 min				
Read the OD at 520 nm.				

CALCULATION OF RESULTS

1. Unit Definition: One unit Tannase activity is defined as the generates 1 µmol of gallic acid per minute in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 0.2 µmol/ml;

V_{Sample}: the volume of reaction sample, 20 µl = 0.02 ml;

$V_{Standard}$: the volume of reaction Standard, 20 µl = 0.02 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

T: the reaction time, 10 minutes.

B. Formula:

a). According to the protein concentration of sample

Tannase activity (U/mg) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

$= 0.02 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

b). According to the weight of sample

Tannase activity (U/mg) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$

$= 0.02 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the cell or bacteria

Tannase activity (U/10^4) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay}) \times T]$

$= 0.02 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times N]$