Tannase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1239

(Version 1.3A)

Detection and Quantification of TannaseActivity inTissue extracts,

Cell lysate, Cell culture media, Other biological fluids Samples.

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

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I. INTRODUCTION

Tannases (tannin acyl hydrolases, EC 3.1.1.20) catalyze the hydrolysis of tannic acid by breaking its ester and depside bonds, releasing glucose and gallic acid. These enzymes are widely used in a number of industrial applications, including the manufacture of instant tea, beer, fruit juices, some wines (by reduction of the adverse effects of tannins in beverages), and treating of tannin-polluting industrial effluents and agricultural wastes. Another important application of tannase is production of gallic acid, used as an important intermediate compound for the chemical synthesis of propyl gallate and trimethoprim, which are important in the food and pharmaceutical industries.

Tannase ActivityColorimetric Microplate Assay Kit is based on hydrolysis of substrate togallic acid. The intensity of the product color, measured at 520 nm, is proportional to the tannaseactivity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 mlx 1	4 °C
Substrate	Powder x 1	4 °C
SubstrateDiluent	4 mlx 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent A Diluent	6 mlx 1	4 °C
Dye Reagent B	4 mlx 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 20 mmol/L of standardstock solution.Keep in dark and store at 4 °C for 1 week or -20°C for 3months after reconstitution.Then dilute to 2 mmol/Lstandard top solutionby adding 100 µlstock solution into 900 µldistilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312mmol/L.

Substrate: Add 4 ml SubstrateDiluent to dissolve before use.Keep in dark and store at 4 °C for 2 weeks or -20°C for 6months after reconstitution.

Dye Reagent A:Add 6 ml Dye Reagent A Diluent to dissolve before use.Keep in dark and store at 4 °C for 1 week or -20°C for 1-2months after reconstitution.

Positive Control:Briefly centrifuge prior to opening. Add 0.1 ml distilled water to dissolve before use, vortex and then centrifuge, take the supernatant intothe plate.Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

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

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Blank	Standard	Positive Control
Substrate	40 µl	40 µl	40 µl	40 µl
Reaction Buffer	40 µl	40 µl	40 µl	40 µl
Sample	20 µl	--	--	--
Distilled water	--	20 µl	--	--
Standard	--	--	20 µl	--
Positive Control	--	--	--	20 µl
Mix, incubate at 30 °C for 10 minutes.				
Dye Reagent A	60 µl	60 µl	60 µl	60 µl
Dye Reagent B	40 µl	40 µl	40 µl	40 µl
Mix, wait for 10 minutes, measured at 520 nm and record the absorbance.				

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: One unit of Tannaseactivity is defined as the enzymeproduces 1μmol of gallic acid per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Blank}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

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

2.1 According to the protein concentration of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N\times(V_{Sample}/ V_{Assay})\times T}(U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times(V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.4 According to the volume of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, mmol/L= μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

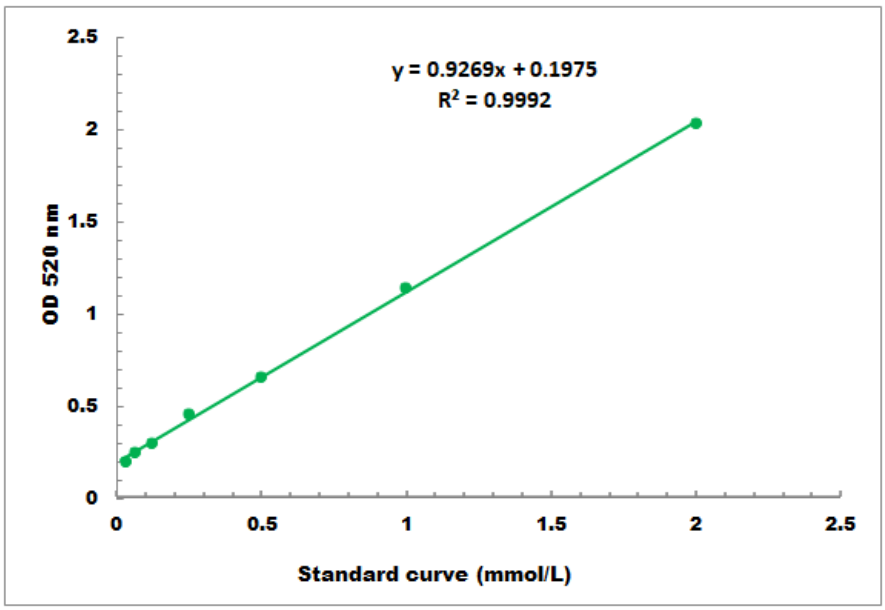
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

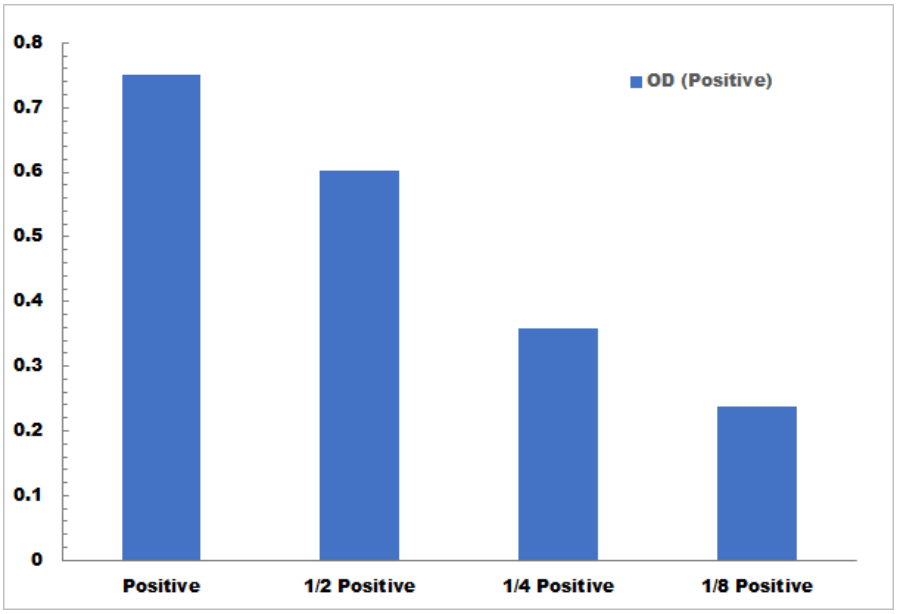
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.02mmol/L -2mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration