TannaseMicroplate Assay Kit

User Manual

Catalog # CAK1239

(Version 1.2A)

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.

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 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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Note:

Substrate:add 4 ml SubstrateDiluent to dissolve before use.

Dye Reagent A:add 6 ml Dye Reagent A Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve, then add 100µl into 900µldistilled water, mix; the concentration will be 2mmol/L.

Positive Control:add 0.1 ml distilled water to dissolve before use, vortex and then centrifuge, take the supernatant intothe plate.

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. 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.

3.For liquid samples

Detect directly, or dilute with Assay Buffer.

V. 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

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

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

2. According to the weight of sample

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

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

3.According to the quantity of cells or bacteria

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

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

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

$C_{Standard}$: the concentration of Standard, 200μmol/L = 0.2μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

V_{Sample}: the volume of sample, 0.02 ml;

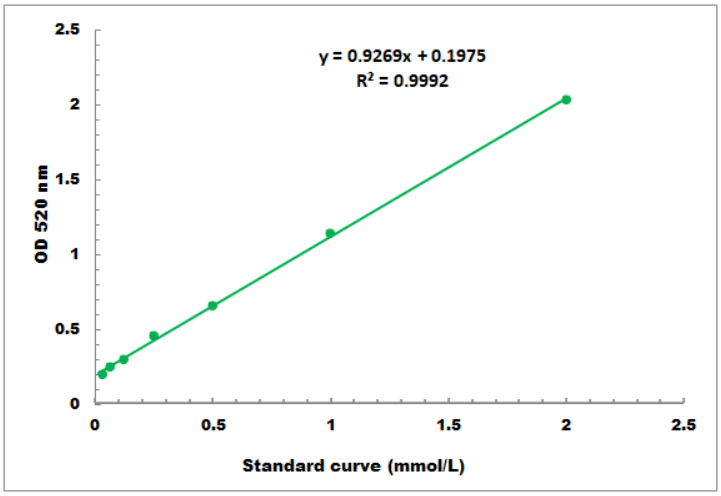
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

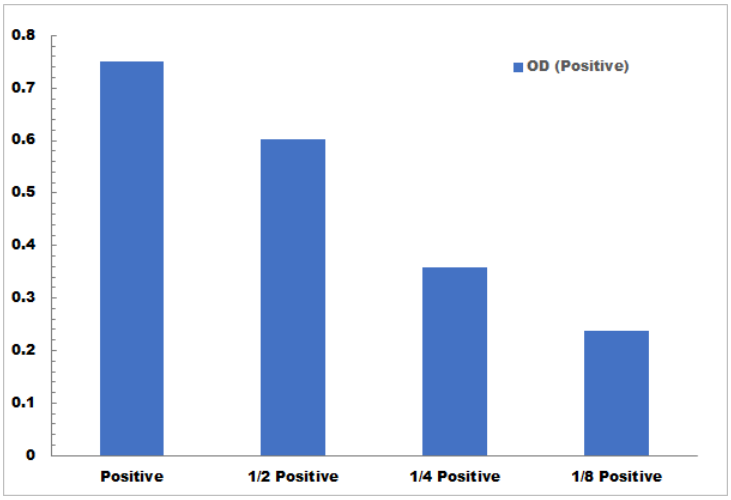
T: the reaction time, 10 minutes.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES