Trypsin Microplate Assay Kit

User Manual

Catalog # ASK1080

Detection and Quantification of Trypsin Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Trypsin (EC 3.4.21.4) is a serine protease found in the digestive system of many vertebrates, where it hydrolyses proteins. Trypsin is produced in the pancreas as the inactive proenzyme trypsinogen. Active trypsin predominantly cleaves peptide chains at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline. It is used for numerous biotechnological processes.

The assay is initiated with the enzymatic catalysis of the BAEE by Trypsin. The enzyme catalysed reaction products BA can be measured at a colorimetric readout at 253 nm.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Substrate: add 1 ml distilled water to dissolve before use.

Standard: add 1 ml Reaction Buffer to dissolve before use, then add 0.25 ml into 0.75 ml Reaction Buffer, mix, the concentration will be 1 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 253 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 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.

2. For tissue samples

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.

3. For serum or plasma samples

Detect directly.

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V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	180 µl	--	200 µl
Substrate	10 µl	--	--
Standard	--	200 µl	--
Distilled water	--	--	--
Sample	10 µl	--	--
Mix, measured at 253 nm and record the sample's absorbance of 10th second and 130th second.			

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VI. CALCULATION

Unit Definition: One unit of Trypsin activity is defined as the enzyme produce 1 μmol BA in the reaction system per minute.

1. According to the protein concentration of sample

$$\text{Trypsin (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 10 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Trypsin (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 10 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Trypsin (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 10 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of liquid sample

$$\text{Trypsin (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 10 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of Standard, 1 mmol/L = 1 μmol/ml;

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

W: the weight of sample, g;

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

$V_{Standard}$: the volume of standard, 0.2 ml;

V_{Sample}: the volume of sample, 0.01 ml;

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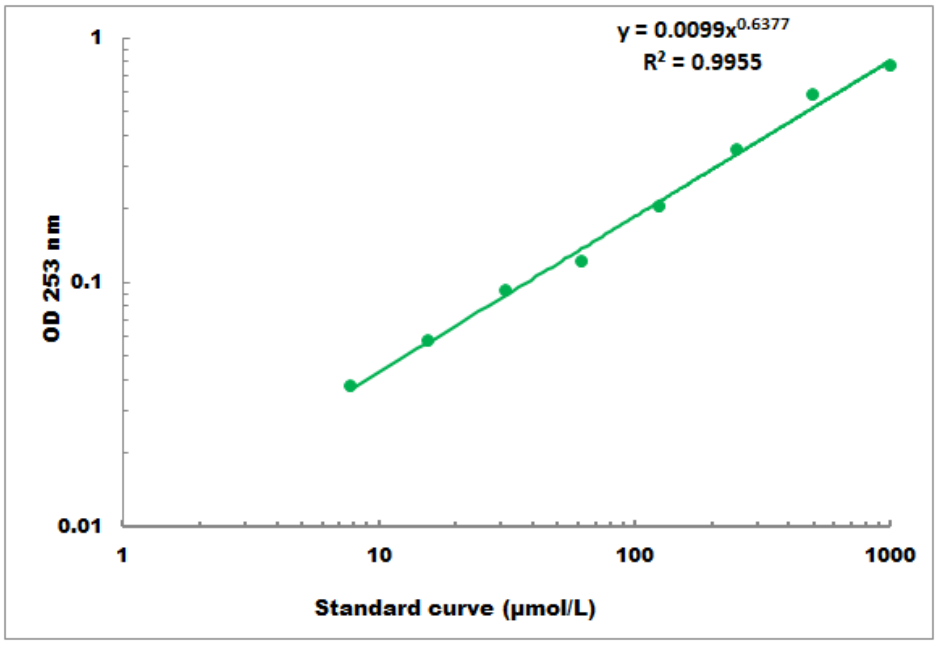
V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

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



Detection Range: 10 μmol/L - 1000 μmol/L

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