



Trypsin Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1080

(Version 2.3D)

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.

Trypsin Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring trypsin activity in a variety of samples. The assay is initiated with the enzymatic catalysis of the BAEE by Trypsin. The enzyme catalysed reaction products p-nitrophenol can be measured at a colorimetric readout at 405 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	1.2 ml x 1	RT
Stop Solution	10 ml x 1	4 °C
Standard (500 µmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8 $\mu\text{mol/L}$.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Substrate Diluent to dissolve before use. Keep in dark and store at 4 °C for 1-2 weeks or -20°C for 2-3 months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use, mix. 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 ml Assay 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 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.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Positive Control	Blank
Reaction Buffer	80 μ l	--	80 μ l	100 μ l
Substrate	10 μ l	--	10 μ l	--
Sample	10 μ l	--	--	--
Positive Control	--	--	10 μ l	--
Mix, put it in the oven, 37°C for 10 minutes.				
Standard	--	100 μ l	--	--
Stop Solution	100 μ l	100 μ l	100 μ l	100 μ l
Mix, record absorbance measured at 405 nm.				

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 Trypsin activity is defined as the enzyme generates 1 μmol of p-nitrophenol per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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, 10⁴

C_{Standard}: the concentration of standard, μmol/L

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

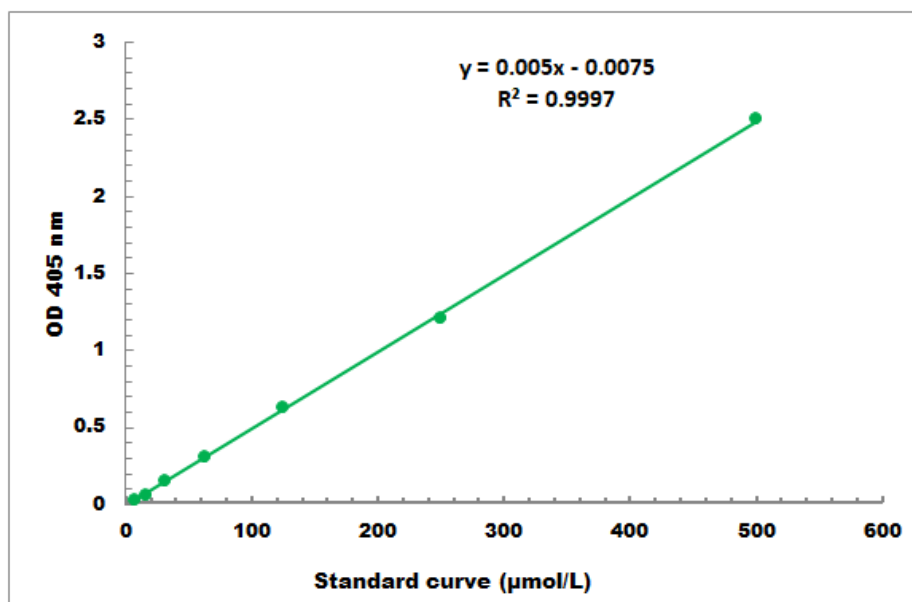
V_{Sample}: the volume of sample in assay procedure, mL

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

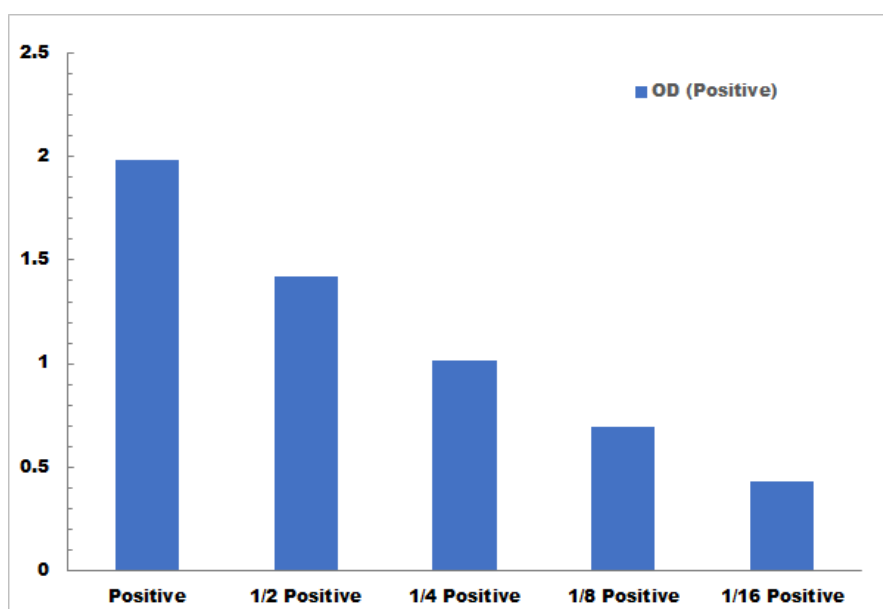
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 5 μmol/L -500μmol/L



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