Pepsin Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1081

(Version 1.3F)

Detection and Quantification ofPepsinActivityin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Pepsin is an enzyme whose zymogen (pepsinogen) is released by the chief cells in the stomach and that degrades food proteins into peptides. Pepsin is a digestive protease, a member of the aspartate protease family.

Pepsin is one of three principal protein-degrading, or proteolytic, enzymes in the digestive system, the other two being chymotrypsin and trypsin. The three enzymes were among the first to be isolated in crystalline form. During the process of digestion, these enzymes, each of which is specialized in severing links between particular types of amino acids, collaborate to break down dietary proteins into their components, i.e., peptides and amino acids, which can be readily absorbed by the intestinal lining. Pepsin is most efficient in cleaving peptide bonds between hydrophobic and preferably aromatic amino acids such as phenylalanine, tryptophan, and tyrosine.

Pepsin ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring pepsin activity in a variety of samples.The assay is initiated with the enzymatic catalysis of the hemoglobin by pepsin. The enzyme catalysed reaction products can be measured at a colorimetric readout at 580 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Diluent	15 ml x 1	4 °C
Stop Solution	10 ml x 1	4 °C
Reaction Buffer	12 ml x 1	4 °C
Dye Reagent	2 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 580 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.Dissolvein1 ml Diluentbefore use, mix, heat at 50 °C water bath to generate 20 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to 5mmol/Lstandard top solutionby adding 0.25 ml stock solution into 0.75 ml Diluent**.** Perform 2-fold serial dilutions of the top standardsolution using Diluentto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.

Substrate: Add 10 ml Diluent to dissolve before use.Store at 4 °C for 1 week or -20°C for 1 month after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 ml distilled waterto dissolve before use.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 10 minutes, 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, wait for 2 hours, 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 Substrateto room temperature before use.

Add following reagents in the microcentrifuge tube:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	20 µl	--	--	--	--
Assay Buffer	--	20 µl	--	--	--
Positive Control	--	--	--	--	20 µl
Substrate	100 µl	100 µl	--	--	100 µl
Mix, put it in water bath of 37°C for 10minutes.					
Stop Solution	100µl	100µl	--	--	100 µl
Mix, centrifuged at 10000g,4 °C for10 minutes, take the supernatant into the microplate.					
Supernatant	60 µl	60 µl	--	--	60 µl
Standard	--	--	60 µl	--	--
Substrate Diluent	--	--	--	60 µl	--
Reaction Buffer	120µl	120µl	120µl	120µl	120µl
Dye Reagent	20 µl	20 µl	20 µl	20 µl	20 µl
Mix,wait for 20 minutes, record absorbance measured at 580 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 Pepsinactivity is defined asthe enzymegenerates 1μmol of Tyrosine per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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

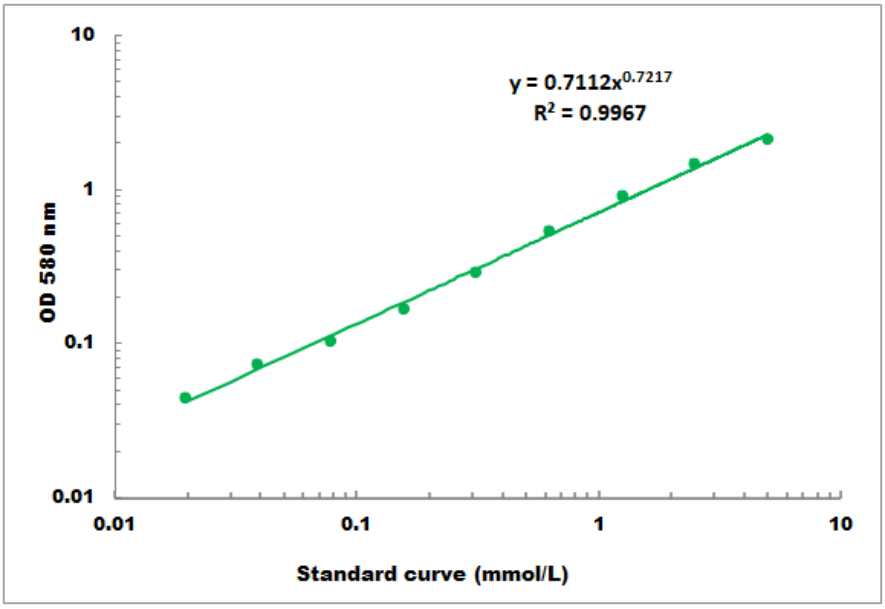
V_{Sample}: the volume of sample in assay procedure, mL

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

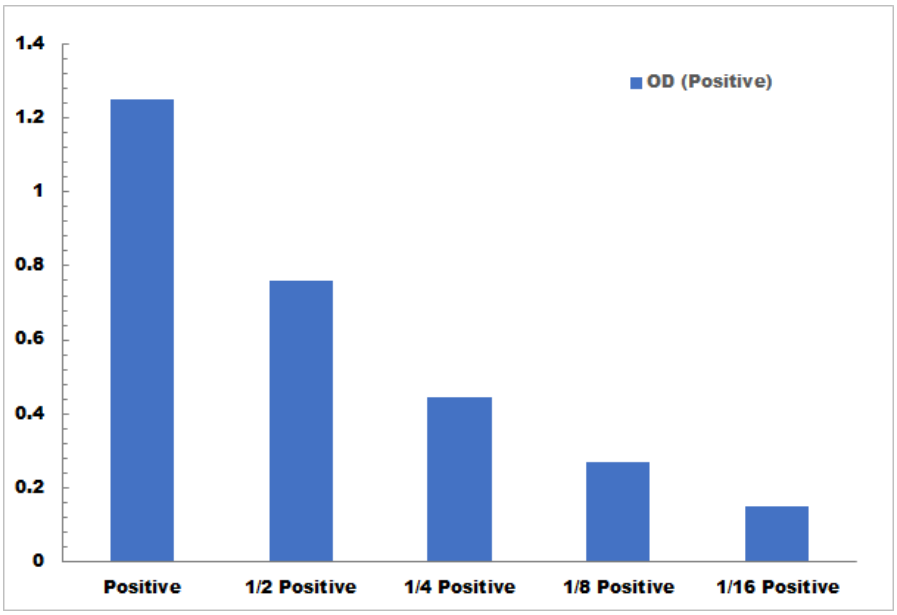
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.01µmol/ml -5µmol/ml



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