Pepsin Microplate Assay Kit

User Manual

Catalog # CAK1081

(Version 1.2F)

Detection and Quantification of Pepsin 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

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 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	30 ml x 4	4 °C
Substrate	Powder x 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
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Note:

Substrate: add 10 ml Diluent to dissolve before use.

Standard: add 1 ml Diluent to dissolve before use, then add 0.25 ml into 0.75 ml Diluent, mix, it will be 5 µmol/ml.

Positive Control: add 0.1 ml distilled water to dissolve before use.

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. 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, 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.

V. ASSAY PROCEDURE

Warm Substrate to 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 10 minutes.					
Stop Solution	100 µl	100 µl	--	--	100 µl
Mix, centrifuged at 10000g, 4 °C for 10 minutes, take the supernatant into the microplate.					
Supernatant	60 µl	60 µl	--	--	60 µl
Standard	--	--	60 µl	--	--
Substrate Diluent	--	--	--	60 µl	--
Reaction Buffer	120 µl	120 µl	120 µl	120 µl	120 µl
Dye Reagent	20 µl	20 µl	20 µl	20 µl	20 µl
Mix, wait for 20 minutes, record absorbance measured at 580 nm.					

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.

VI. CALCULATION

Unit Definition: One unit of Pepsin activity is defined as the enzyme generates 1 μmol of Tyrosine per minute.

1. According to the protein concentration of sample

$$\text{Pepsin (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T \times 11$$

$$= 5.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Pepsin (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T \times 11$$

$$= 5.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Pepsin (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T \times 11$$

$$= 5.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$\text{Pepsin (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T \times 11$$

$$= 5.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 5 μmol/ml;

$V_{Standard}$: the volume of standard, 0.06 ml;

V_{Sample}: the volume of sample, 0.06 ml;

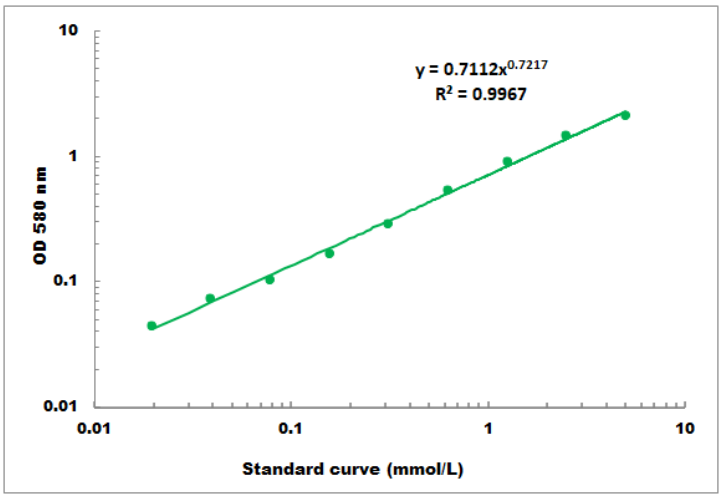
V_{Assay}: the volume of Assay buffer, 1 ml;

N: the quantity of cell or bacteria, $N \times 10^4$;

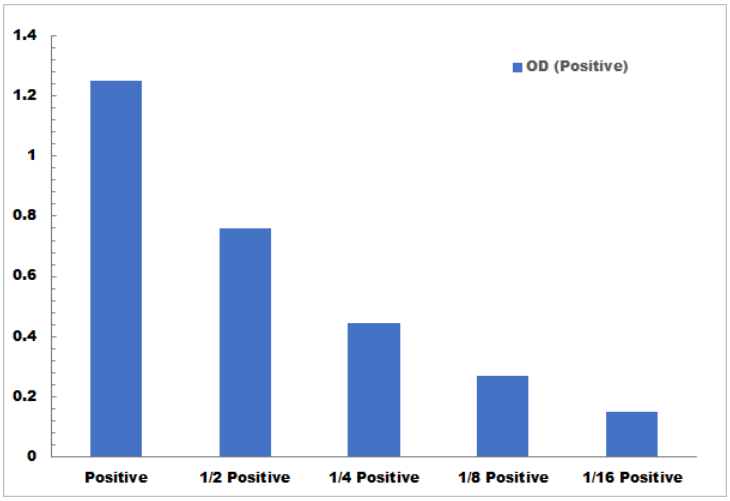
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



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



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

VIII. TECHNICAL SUPPORT

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

IX. NOTES