Pepsin Assay Kit

Catalog No.: abx298847

Size: 100 Assays

Storage: Store the Positive Control at -20°C and all other kit components in the dark at 4°C.

Application: For quantitative detection of Pepsin activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

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

Introduction: Pepsin is an endopeptidase primarily found in the chief cells of the stomach lining. It is activated from its proenzyme pepsinogen by hydrochloric acid in the gastric fluid. Pepsin preferentially cleaves peptide bonds between hydrophobic and aromatic amino acids such as phenylalanine, tryptophan, and tyrosine.

Pepsin catalyzes the hydrolysis of hemoglobin. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 580 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 12 ml
4. Diluent Buffer: 15 ml
5. Stop Solution: 10 ml
6. Dye Reagent: 2 ml
7. Standard: 1 vial
8. Substrate: 1 vial
9. Positive Control: 1 vial

Materials Required But Not Provided

1. Microplate reader (580 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 10 ml of Diluent Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of Diluent Buffer to the Standard vial and mix thoroughly. Add 250 µl of this solution to 750 µl of Diluent Buffer to prepare a 1 ml Standard Solution with concentration 5 µmol/ml.

- **Positive Control Solution**
  Add 1 ml of distilled water to the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use.

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2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer, then allow to stand for 2 hours. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

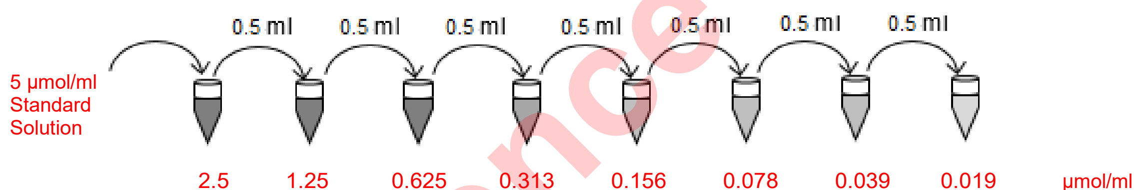
- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 8 tubes with 2.5 µmol/ml, 1.25 µmol/ml, 0.625 µmol/ml, 0.313 µmol/ml, 0.156 µmol/ml, 0.078 µmol/ml, 0.039 µmol/ml and 0.019 µmol/ml. Aliquot 0.5 ml of Assay Buffer into each tube. Add 0.5 ml of 5 µmol/ml standard solution to the 1st tube (2.5 µmol/ml) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, control, and positive control microcentrifuge tubes. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of sample to the sample tubes.
4. Add 20 µl of Assay Buffer to the control tubes.
5. Add 20 µl of Positive Control Solution to the positive control tubes.
6. Add 100 µl of Substrate Solution to all tubes. Mix thoroughly.
7. Incubate at 37°C in a water bath for 10 minutes.
8. Add 100 µl of Stop Solution to all tubes. Mix thoroughly.
9. Centrifuge all tubes at 10,000 × g at 4°C for 10 minutes.
10. Set the sample, standard, control and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
11. Add 60 µl of sample supernatant from the sample tubes in step 9 to the sample wells.
12. Add 60 µl of control supernatant from the control tubes in step 9 to the control wells.
13. Add 60 µl of positive control supernatant from the positive control tubes in step 9 to the control wells.
14. Add 60 µl of Diluent Buffer to the blank wells.
15. Add 60 µl of prepared standard solutions to the standard wells.
16. Add 120 µl of Reaction Buffer to all wells.
17. Add 20 µl of Dye Reagent to all wells.
18. Tap the plate gently to mix. Allow to stand for 20 minutes.
19. Read and record absorbance at 580 nm.

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C. Calculations

One unit of Pepsin activity is defined as the amount of enzyme required to produce 1 μmol of tyrosine per minute.

Pepsin activity per mg of protein:

$$\text{Pepsin (U/mg)} = 11 \times \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times C_{Protein} \times T} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} = \frac{5.5}{C_{Protein}} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}}$$

Pepsin activity per g of sample:

$$\text{Pepsin (U/g)} = 11 \times \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times W \times T} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} = \frac{5.5}{W} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}}$$

Pepsin activity per 10^4 cells or bacteria:

$$\text{Pepsin (U/10}^4\text{ cells)} = 11 \times \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times N \times T} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} = \frac{5.5}{N} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}}$$

Pepsin activity per ml of sample:

$$\text{Pepsin (U/ml)} = 11 \times \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times T} \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} = 5.5 \times \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Protein}$ Concentration of protein (in mg/ml)

$C_{Standard}$ Concentration of highest standard (5 μmol/ml)

T Reaction time (10 minutes)

W Weight of the sample (in g)

N Number of cells or bacteria (× 10^4)

V_{Assay} Volume of assay buffer (1 ml)

V_{Sample} Volume of sample (0.06 ml)

$V_{Standard}$ Volume of standard (0.06 ml)

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