Acidic Protease Assay Kit

Catalog No.: abx298843

Size: 100 Assays

Storage: Store all components at 4°C in the dark for up to 6 months.

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

Detection Range: 0.05 mmol/L – 5 mmol/L

Introduction: Acidic Proteases (ACP) are bacterial enzymes that exhibit proteolytic activity at acidic pH. They are widely used in many biotechnological processes, such as alcohol and distilled spirit processing, and animal feed production.

Abbexa's Acidic Protease Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Acidic Protease activity, by measuring the enzymatic catalysis of casein. The concentration of the reaction products of the assay can be determined by reading the absorbance of 660 nm, from which the Acidic Protease activity can be calculated.

Kit components

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

Materials Required But Not Provided

1. Microplate reader (660 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 4 ml of Assay Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.
- **Positive Control Solution**
  Add 100 µl of Assay Buffer to the Positive Control vial and mix thoroughly. Ensure that the Positive Control has completely dissolved prior to use.
- **Standard Solution**
  Add 1 ml of Standard Diluent to the Standard vial and mix thoroughly. Take 500 µl of this solution and add to 500 µl of distilled water to prepare a 1 ml Standard Solution with concentration 5 mmol/L.

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 on ice. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

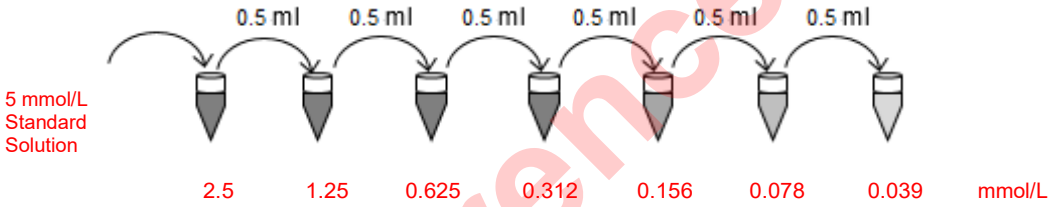
- **Serum and plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

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

1. Label 7 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.312 mmol/L, 0.156 mmol/L, 0.078 mmol/L and 0.039 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set sample, positive control, standard, control and blank microcentrifuge tubes and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 40 µl of sample to each sample tube.
4. Add 40 µl of Positive Control solution to each positive control tube.
5. Add 40 µl of prepared standard to each standard tube.
6. Add 40 µl of Standard Diluent to each blank tube.
7. Add 40 µl of Assay Buffer to each standard tube, control tube and blank tube.
8. Add 40 µl of Substrate Solution to each sample tube, positive control tube and control tube.
9. Mix each tube, then incubate at 40°C for 15 minutes.
10. Add 120 µl of Stop Solution to each tube.
11. Mix each tube, then centrifuge at 10,000 × g for 10 minutes. If any precipitate is observed in the tube, centrifuge at 4000 × g for 5 minutes.
12. Set sample, positive control, standard, control and blank wells on the microplate and record their positions. We recommend setting up each standard and sample in duplicate.
13. For each tube, add 100 µl of supernatant (from step 10) to the corresponding well on the microplate.
14. Add 60 µl of Reaction Buffer to each well.
15. Add 40 µl of Dye Reagent to each well.
16. Tap the plate gently to mix. Allow to stand at room temperature for 20 minutes.
17. Read and record the absorbance at 660 nm.

C. Calculations

One unit of Acidic Protease activity is defined as the amount of enzyme required to generate 1 µmol of tyrosine per minute.

1. Acidic Protease activity per mass of protein (in mg):

$$\mathbf{ACP\ (U/mg\ protein)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{C_{Protein} \times V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}} = \frac{\mathbf{333.3}}{\mathbf{C_{protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}}$$

2. Acidic Protease activity per weight of sample (in g):

$$\mathbf{ACP\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{W \times V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}} = \frac{\mathbf{333.3}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}}$$

3. Acidic Protease activity per 10^4 cells or bacteria:

$$\mathbf{ACP\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{N \times V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}} = \frac{\mathbf{333.3}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}}$$

4. Acidic Protease activity per volume of sample (in ml):

$$\mathbf{ACP\ (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}} = \mathbf{333.3} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Control}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (mg/ml)
$\mathbf{C_{Standard}}$	Concentration of the highest standard (5 mmol/L = 5000 nmol/ml)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{T}$	Reaction time (15 minutes)
$\mathbf{W}$	Weight of sample (g)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.04 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.04 ml)