Acidic Protease Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1077

(Version 1.6E)

Detection and Quantification of Acidic Protease (ACP) 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.

I. INTRODUCTION

Acidic protease (ACP) can act on interior bond -C-NH- to hydrolyze the proteins of vegetable and animal in acid environment to form small peptides and amino acidics. The addition of this product to the processing of alcohol and distilled spirit can promote the growth of yeast to accelerate fermentation speed and improve alcohol productivity. In feedstuff industry, this product can be used as an additive by combined with other enzymes (such as cellulase, pectinase and β-glucanase, etc) for the preparation of complex feedstuff enzymes. It can further improve the nutrition value of feedstuff to accelerate the growth and increase the weight of livestock and poultry.

Acidic Protease Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring acidic protease activity in a variety of samples. The assay is initiated with the enzymatic catalysis of casein by ACP in acid environment. The enzyme catalysed reaction products can be measured at a colorimetric readout at 660 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
Stop Solution	12 ml x 1	4 °C
Reaction Buffer	6 ml x 1	4 °C
Dye Reagent	4 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Standard Diluent	5 ml x 1	4 °C
Positive Control	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 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. Dissolve in 1 ml Standard Diluent before use, mix, heat at 50 °C water bath to generate 10 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 5 mmol/L standard top solution by adding 500 µl stock solution into 500 µl Standard Diluent**.** Perform 2-fold serial dilutions of the top standard solution using Standard Diluent to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

Substrate: Add 4 ml Assay Buffer to dissolve before use. Store at 4 °C for 3-4 days or -20°C for 1-2 months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 100 µl Assay Buffer to 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 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

Add following reagents into the microcentrifuge tubes:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Sample	40 µl	--	--	--	--
Positive Control	--	--	40 µl	--	--
Standard	--	--	--	40 µl	--
Standard Diluent	--	--	--	--	40 µl
Assay Buffer	--	40 µl	--	40 µl	40 µl
Substrate	40 µl	40 µl	40 µl	--	--
Mix, put it in the oven, 40 °C for 15 minutes.					
Stop Solution	120 µl	120 µl	120 µl	120 µl	120 µl
Mix, centrifuged at 10,000g 4 °C for 10 minutes, add the supernatant into the microplate.					
Supernatant	100 µl	100 µl	100 µl	100 µl	100 µl
Reaction Buffer	60 µl	60 µl	60 µl	60 µl	60 µl
Dye Reagent	40 µl	40 µl	40 µl	40 µl	40 µl
Mix, incubate at room temperature for 20 minutes, measured at 660 nm and record the absorbance***.					

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.

*** If there is any precipitation or floc before read out, please centrifuged it in the microcentrifuge tubes at 4000g for 5 minutes, then add the supernatant into the plate, measured at 660 nm.

VII. CALCULATION

Unit Definition: One unit of ACP activity is the enzyme that generates 1 μmol of Tyrosine per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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

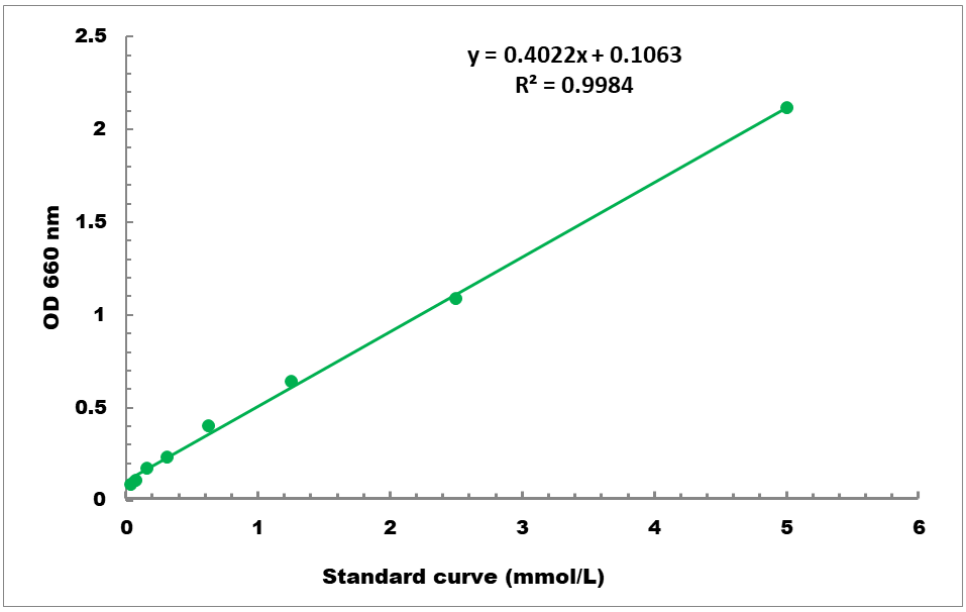
V_{Sample}: the volume of sample in assay procedure, mL

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

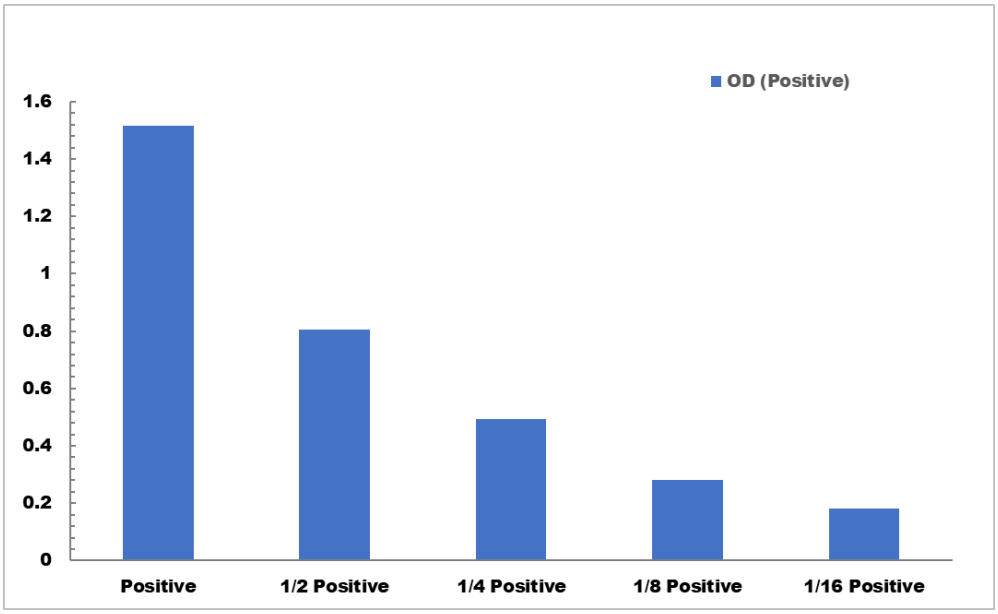
T: the reaction time, minute

VIII. TYPICAL DATA

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



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



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