Acid Protease(ACP) Activity Assay Kit

Product Code: 112893

Acid Protease (ACP) Activity Assay Kit - Micromethod

Product Overview

Acid protease (ACP) is an enzyme that catalyzes protein hydrolysis under acidic conditions. It is commonly used in alcohol fermentation, beer brewing, fur softening, fruit wine clarification, soy sauce brewing, and feed applications.

In this assay, ACP hydrolyzes casein under acidic conditions to produce tyrosine. Under alkaline conditions, tyrosine reduces phosphomolybdic acid compounds to form tungsten blue, which has a characteristic absorption peak at 680 nm. ACP activity is calculated from the increase in absorbance at 680 nm.

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Package Contents

Component Code	Component	Quantity
BR5000197.1	Reagent I A	1 vial
BR5000197.2	Reagent I B	1 vial
BR5000197.3	Reagent II	1 bottle
BR5000197.4	Reagent III	1 bottle
BR5000197.5	Reagent IV	1 bottle
BR5000197.6	Reagent V	1 bottle
BR5000197.7	Standard	1 vial
BR5000197.m	Manual	1 copy

Kit size: 100T

Quality and Safety

Material	Quality Standard	Main Toxicity
Reagent I A	--	--
Reagent I B	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Reagent V	--	--
Standard	--	--

Transportation and Storage

Transport with ice packs.

Store at 2-8°C, protected from light. Shelf life: 180 days.

Instructions for Use

1. Reagent Preparation

1. Prepare Reagent I fresh before use by mixing Reagent I A : Reagent I B : distilled water = 90 μL : 20 μL : 21 mL. Use

immediately after preparation.
2. Before use, add 4 mL distilled water to dissolve one reagent component.
3. Before use, add 10 mL Reagent I to Reagent III. Dissolve by magnetic stirring in a boiling water bath. A layer of plastic wrap may be used to cover the beaker. Watch carefully to prevent complete evaporation of the water. Heating usually takes 15-30 minutes. This reagent is supersaturated; any particulate insolubles remaining after thorough mixing do not affect use.
4. Before use, add 50 mL distilled water to dissolve one reagent component.

2. Crude Enzyme Extract Preparation

1. Tissue: use a tissue mass (g) to Reagent I volume (mL) ratio of 1:5-10. Recommended: weigh about 0.1 g tissue, add 1 mL Reagent I, homogenize in an ice bath, centrifuge at 8000g and 4°C for 10 minutes, then collect the supernatant as the crude enzyme extract.
2. Bacteria and fungi: use a cell number (10^4cells) to Reagent I volume (mL) ratio of 500-1000:1. Recommended: use 500 x 10^4cells with 1 mL Reagent I, disrupt ultrasonically in an ice bath at 300 W with 3 s sonication and 7 s intervals for a total of 3 minutes, then centrifuge at 8000g and 4°C for 10 minutes. Collect the supernatant and keep it on ice for testing.
3. Serum or culture medium: measure directly.

3. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for 30 minutes. Set the wavelength to 680 nm and zero the instrument with distilled water.
2. Place Reagent II, Reagent III, and Reagent IV in a 30°C water bath for 30 minutes before use.
3. Control tube: add 20 μL crude enzyme extract and 40 μL Reagent II to one EP tube. Mix well and incubate in a 30°C water bath for 10 minutes. Add 40 μL Reagent III, mix well, then centrifuge at 8000g and 4°C for 10 minutes. Transfer 40 μL supernatant to a new tube, add 200 μL Reagent IV and 40 μL Reagent V, mix well, and incubate in a 30°C water bath for 20 minutes. Transfer 200 μL to a micro glass cuvette or 96-well plate and measure absorbance at 680 nm. Record as $A_{control}$.
4. Assay tube: add 20 μL crude enzyme solution and 40 μL Reagent III to one tube. Mix well and incubate in a 30°C water bath for 10 minutes. Add 40 μL Reagent II, then centrifuge at 8000g and 4°C for 10 minutes. Transfer 40 μL supernatant to a new tube, add 200 μL Reagent IV and 40 μL Reagent V, mix well, and incubate in a 30°C water bath for 20 minutes. Transfer 200 μL to a micro glass cuvette or 96-well plate and measure absorbance at 680 nm. Record as $A_{measured}$.
5. Blank tube: add 40 μL distilled water, 200 μL Reagent IV, and 40 μL Reagent V to one tube. Mix well and incubate in a 30°C water bath for 20 minutes. Transfer 200 μL to a micro glass cuvette or 96-well plate and measure absorbance at 680 nm. Record as A_{blank}.
6. Standard tube: add 40 μL standard solution, 200 μL Reagent IV, and 40 μL Reagent V to one tube. Mix well and incubate in a 30°C water bath for 20 minutes. Transfer 200 μL to a micro glass cuvette or 96-well plate and measure absorbance at 680 nm. Record as $A_{standard}$.

For the assay tube, add Reagent III first and then Reagent II. The blank tube and standard tube only need to be measured once.

Calculation of Acid Protease Activity

1. Based on Protein Concentration

Unit definition: at 30°C, one unit is the amount of enzyme that produces 1 nmol tyrosine per minute per mg protein.

ACP activity (nmol/min/mg prot) = $C_{standard}$x ($A_{measured}$- $A_{control}$) / ($A_{standard}$- A_{blank}) x $V_{total\ reaction}$/ (Cpr x V1) / T

ACP activity (nmol/min/mg prot) = 125 x ($A_{measured}$- $A_{control}$) / ($A_{standard}$- A_{blank}) / Cpr

2. Based on Sample Mass

Unit definition: at 30°C, one unit is the amount of enzyme in 1 g sample that produces 1 nmol tyrosine per minute.

ACP activity (nmol/min/g fresh weight) = $C_{standard}$x ($A_{determination}$- $A_{control}$) / ($A_{standard}$- A_{blank}) x $V_{total\ reaction}$/ (W x V1 / V2) / T

ACP activity (nmol/min/g fresh weight) = 125 x ($A_{determination}$- $A_{control}$) / ($A_{standard}$- A_{blank}) / W

3. Based on Cell Number

Unit definition: at 30°C, one unit is the amount of enzyme in 10^4cells that produces 1 nmol tyrosine per minute.

ACP activity (nmol/min/10^4cells) = $C_{standard}$x ($A_{determination}$- $A_{control}$) / ($A_{standard}$- A_{blank}) x $V_{total\ reaction}$/ (cell number x V1 / V2) / T

ACP activity (nmol/min/10^4cells) = 125 x (A_{assay}- $A_{control}$) / ($A_{standard}$- A_{blank}) / cell count

4. Based on Liquid Volume

Unit definition: at 30°C, one unit is the amount of enzyme in 1 mL sample that produces 1 nmol tyrosine per minute.

ACP activity (nmol/min/mL) = $C_{standard}$x (A_{assay}- $A_{control}$) / ($A_{standard}$- A_{blank}) x $V_{total\ reaction}$/ V1 / T

ACP activity (nmol/min/mL) = 125 x (A_{assay}- $A_{control}$) / ($A_{standard}$- A_{blank})

Parameter Definitions

Symbol	Definition	Value
$C_{standard}$	Standard concentration	0.25 μmol/mL
V_{total}	Total volume of the enzymatic reaction	0.1 mL
Cpr	Protein concentration of the crude enzyme solution	mg/mL
V1	Volume of crude enzyme solution added to the reaction system	0.02 mL
V2	Total volume of the extract	1 mL
T	Catalytic reaction time	10 min
W	Sample mass	g

Precautions

1. Prepare only the amount of Reagent I needed for immediate use. Do not use it after white flocculent precipitates appear.
2. Freshly prepared reagents should be stored at 4°C and used within 3 days.
3. Because each assay tube requires a corresponding control tube, the 100T kit can test 48 samples.

Visual Reference