Total Protease Activity Assay Kit

Cat. No. KITG-040 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Protease activity is commonly determined using natural and synthetic substrates. Various proteases can hydrolyze natural substrates, and the activity measured using casein as substrate represents the total protease activity. This assay kit utilizes the ability of total protease to catalyze the hydrolysis of azocasein. The reaction product has an absorption maximum at 366 nm. The change in absorbance is measured to determine total protease activity.
Description	This Total Protease Activity Assay Kit provides a convenient and sensitive method for measuring total protease activity using azocasein as substrate. The kit is suitable for detecting protease activity in tissue samples, bacterial/cell samples, and liquid samples. The assay is based on the hydrolysis of azocasein by proteases, producing a colored product that can be quantified by measuring absorbance at 366 nm using a microplate reader. The kit includes all necessary components for performing the assay and can accommodate 48 samples.
Form	Liquid and Powder
Applications	Measurement of total protease activity in tissue homogenates; Detection of protease activity in bacterial and cell lysates; Determination of protease activity in liquid samples; Quality control of protease preparations; Research on protease-related physiological and pathological processes
Notes	It is recommended to select 2 samples for pre-testing before formal experiments to understand the sample conditions and familiarize with the experimental procedure,

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	avoiding waste of samples and reagents. This operation procedure is suitable for the vast majority of routine samples. Experimental conditions can be appropriately adjusted according to the actual sample status. For special sample types, technical support can provide customized optimization recommendations. For research use only. Not for use in diagnostic or therapeutic procedures.
Storage	Store at 4°C.
Shipping	Shipped with ice packs
Reaction conditions	Reaction temperature: 30°C Reaction time: 60 min Measurement wavelength: 366 nm Incubation after adding Reagent III: 5 min on ice, then room temperature Centrifugation: 12000 rpm for 5 min at room temperature
Unit definition	One unit is defined as the amount of enzyme that causes a change of 1 absorbance unit per hour in the reaction system. For tissue samples: 1 azocasein unit per hour per gram of fresh tissue. For protein concentration: 1 azocasein unit per hour per milligram of protein. For bacterial/cell samples: 1 azocasein unit per hour per 10^4 cells. For liquid samples: 1 azocasein unit per hour per milliliter of liquid.
Usage	Kit Components (48 Tests): Extraction Buffer: Liquid, 60 mL × 1 bottle (Store at 4°C) Reagent I: Powder, 1 bottle (Store at 4°C). Before use, shake to settle the powder to the bottom, then add 13 mL of distilled water and mix well for use. Reagent II: Liquid, 12 mL × 1 bottle (Store at 4°C) Reagent III: Liquid, 36 mL × 1 bottle (Store at 4°C) Materials Required but Not Provided: Microplate reader 96-well microplate Benchtop centrifuge Adjustable pipettes Balance Mortar and pestle Ice and distilled water Sample Preparation: 1. Tissue Samples: Take approximately 0.1 g of tissue, add 1 mL of Extraction Buffer, and homogenize on ice. Centrifuge at 4°C, 12000 rpm for 10 min. Collect the supernatant and keep on ice for testing. Note: If increasing sample volume, extract according to the ratio of tissue mass (g): Extraction Buffer volume

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

(mL) = 1:5~10. 2. Bacterial/Cell Samples: Collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant. Take approximately 5 million bacteria or cells, add 1 mL of Extraction Buffer, and sonicate on ice (power 200W, ultrasound 3s, interval 10s, repeat 30 times). Centrifuge at 4°C, 12000 rpm for 10 min. Collect the supernatant and keep on ice for testing. Note: If increasing sample volume, extract according to the ratio of bacterial/cell count (10^4): Extraction Buffer volume (mL) = 500~1000:1. 3. Liquid Samples: Test directly. If turbid, centrifuge and take the supernatant for testing. Assay Procedure: 1. Preheat the microplate reader for more than 30 min, and set the wavelength to 366 nm. 2. Thaw all reagents to room temperature or incubate in a 25°C water bath for 20 min. 3. Add the following reagents sequentially into EP tubes (μL): Test Tube: - Sample: 120 μL - Reagent I: 120 μL - Reagent II: 120 μL Mix well, incubate at 30°C for 60 min. - Reagent III: 360 μL Mix immediately, place on ice for 5 min. Then centrifuge at room temperature, 12000 rpm for 5 min. Directly take 200 μL of supernatant into a 96-well plate and measure absorbance at 366 nm. Control Tube: - Sample: 120 μL - Reagent II: 120 μL Mix well, incubate at 30°C for 60 min. - Reagent III: 360 μL - Reagent I: 120 μL Mix immediately, place on ice for 5 min. Then centrifuge at room temperature, 12000 rpm for 5 min. Directly take 200 μL of supernatant into a 96-well plate and measure absorbance at 366 nm. Calculate ΔA = A_test - A_control (each sample serves as its own control). Data Analysis: 1. Calculation by Fresh Tissue Weight: Definition: One azocasein unit is defined as the amount of enzyme that causes a change of 1 absorbance unit per hour per gram of fresh tissue in the reaction system. Total Protease (azocasein unit/h/g fresh weight) = ΔA ÷ 1 ÷ (W × V1 ÷ V) ÷ T = 8.3 × ΔA ÷ W 2. Calculation by Protein Concentration: Definition: One azocasein unit is defined as the amount of enzyme that causes a change of 1 absorbance unit per hour per milligram of protein in the reaction system. Total Protease (azocasein unit/h/mg prot) = ΔA ÷ 1 ÷ (V1 × Cpr) ÷ T = 8.3 × ΔA ÷ Cpr 3. Calculation by Bacterial/Cell Count: Definition: One azocasein unit is defined as the amount of enzyme that causes a change of 1 absorbance unit per hour per 10^4 cells in the reaction system. Total

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	Protease (azocasein unit/h/10^4 cell) = ΔA ÷ 1 ÷ (500 × V1 ÷ V) ÷ T = 0.02 × ΔA ÷ Cpr 4. Calculation by Liquid Volume: Definition: One azocasein unit is defined as the amount of enzyme that causes a change of 1 absorbance unit per hour per milliliter of liquid in the reaction system. Total Protease (azocasein unit/h/mL) = ΔA ÷ 1 ÷ V1 ÷ T = 8.3 × ΔA Where: V = Volume of Extraction Buffer added, 1 mL V1 = Volume of sample added, 0.12 mL W = Sample mass, g T = Reaction time, 60 min = 1 h 500 = Cell count, 10^4 Cpr = Sample protein concentration, mg/mL (determined using BCA protein assay kit)

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA