Jul 16, 2020 Version 2

Enzymatic Assay of Protease Using Azocasein as Substrate V.2

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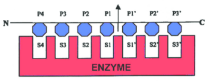

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Protocol status: Working
We use this protocol and it's working

Created: June 19, 2020

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Protocol Integer ID: 38382

Keywords: Enzyme, substrate, kinetic, enzymology, protein

Materials

MATERIALS

- Calcium chloride **Merck Millipore Catalog #**1.02378.0500
- Trichloroacetic acid (TCA) **Sigma – Aldrich Catalog #**T6399
- Sodium hydroxide **Sigma – Aldrich Catalog #**S8045
- Trizma® base **Sigma Aldrich Catalog #**T4661
- Azocasein **Catalog #**A2765

Safety warnings

Wear personal protective equipment: gloves, lab coat and mask.

Before start

Organize your workspace

Make sure all solutions and equipment are available.

Reagent Preparation

1

- 100 mM Tris-HCl buffer, pH 8.0, 20 mM $CaCl_2$, at 37 °C.

- 2.0% (w/v) Azocasein Solution

Heat gently (do not boil) to 50 - 60 °C for 10 min with stirring.
Adjust the pH to 8.0 at 37 °C, if necessary, with either 1.0 M NaOH or 1.0 M HCl.

- 110 mM Trichloroacetic Acid Reagent (TCA). Dilute with deionized water.

- 500 mM Sodium Hydroxide (NaOH) Solution. Prepare in deionized water.

Check how many samples will be analyzed to calculate the required volume of each solution to be prepared.

Procedure

2

Pipette (in microliters) the following reagents into 2.0 mL microtubes.

		Blank	Test
	Tris-HCl buffer	750 µL	450 µL
	Azocasein	750 µL	750 µL
	Mix and equilibrate to the at desired temperature. Then add:	*	
	Sample (enzyme source)	-	300 µL
	Mix and incubate at desired temperature for exactly 30 min.	*	
	Remove a 1 mL aliquot from both (test and blank) solutions and place into 2.0 mL microtubes. Then add:		
	TCA	1000 µL	1000 µL
	Centrifuge at 20,000 g for 10 min. Remove a 1 mL aliquot from supernatant (test and blank) and place into 2.0 mL microtubes. Then add:	*	
	NaOH	1000 µL	1000 µL
	Mix and transfer the Test and Blank solutions to suitable cuvettes. Measure the A440nm for Test and Blank using a spectrophotometer.	*	

Calculation

3 $\Delta A_{440nm} = A_{440nm}Test - A_{440nm}Blank$