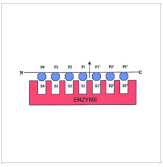

Version 2

Jul 16, 2020

Enzymatic Assay of Protease Using Azocasein as Substrate V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bhqnj5ve

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KEYWORDS

Enzyme, substrate, kinetic, enzymology, protein

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MATERIALS

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

SAFETY WARNINGS

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

BEFORE STARTING

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

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