VERSION 2

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

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Enzymatic Assay of Protease Using Azocasein as Substrate V.2

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MATERIALS

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☒ 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 **Contributed by users Catalog #A2765**

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

Organize your workspace

Make sure all solutions and equipment are available.

Reagent Preparation

- 1
 - 100 mM Tris-HCl buffer, pH 8.0, 20 mM CaCl₂, 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 A _{440nm} for Test and Blank using a spectrophotometer.	*	

Calculation

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