NOV 14, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.9a4h2gw

Protocol Citation: Neilier Junior 2019. Enzymatic Assay of Trypsin Inhibition.

protocols.io
<https://dx.doi.org/10.17504/protocols.io.9a4h2gw>

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

Created: Nov 13, 2019

Last Modified: Nov 14, 2019

PROTOCOL integer ID:
 29756

Keywords: Enzymatic assay,
 Trypsin inhibitor

Enzymatic Assay of Trypsin Inhibition

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ABSTRACT

This protocol has been standardized for analysis of protease inhibitors in soybean leaf extract, but can be easily adjusted for other biological samples.

MATERIALS

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⊗ Tris **Contributed by users**

⊗ DMSO **Sigma Aldrich Catalog #D1435**

⊗ Calcium Chloride **Sigma Aldrich Catalog #C4904**

⊗ Trypsin from bovine pancreas **Sigma-aldrich Catalog #T8003**

⊗ N α -Benzoyl-L-arginine 4-nitroanilide hydrochloride (L-BApNA) **Sigma-aldrich Catalog #B3279**

BEFORE START INSTRUCTIONS

Check that all reagents and equipment are available. Plan the experiment!

Prepare the solutions and the workspace

1 Preparation of solutions

Trypsin solution:

Dilute 1.25 mg of bovine trypsin in 1 mL of water pH 3.0 (adjust with HCl).

L-BAPNA stock solution:

Dilute 130.47 mg L-BAPNA in 5 mL DMSO (concentration: 6.0×10^{-2} M). Store at -20 °C and protected from light.

L-BAPNA usage solution (freshly prepared):

Dilute 200 mL of stock solution in 10 mL of 100 mM Tris-HCl buffer, pH 8.2 and 20 mM CaCl₂.

Protect from light.

2 Separate three microtubes and name them "**blank**", "**control** (uninhibited test)" and "**test** (inhibited test)".

Pipette the following reagents.

Blank: 500 µL BAPNA usage solution and 500 µL buffer.

Control: 100 µL trypsin solution and 400 µL buffer.

Test: 100 µL enzyme, 100 µL leaf extract (source of inhibitors) and 300 µL buffer.

3 Mix the three microtubes by inversion and equilibrate to 25°C for 5 min

Zero spectrophotometer with **blank** content at 410 nm

4 To the **control** microtube, add 500 µL of the BAPNA usage solution

Immediately mix by inversion and mark the time and pour the contents into a cuvette. After 30 s of reaction onset, monitor readings at 410 nm for 120 s

5 Add 500 µL of the BAPNA usage solution to the **test** tube

Immediately mix by inversion and mark the time and pour the contents into a cuvette. After 30 s of reaction onset, monitor readings at 410 nm for 120 s

6 Calculations

$$\% \text{ Inhibition} = (\Delta A_{410 \text{ nm}}^{\text{control}} - \Delta A_{410 \text{ nm}}^{\text{test}}) * 100 / (\Delta A_{410 \text{ nm}}^{\text{control}} - A_{410 \text{ nm}}^{\text{blank}})$$

or

Trypsin Inhibitor Units / mL = $(\Delta A_{410 \text{ nm}}^{\text{control}} - \Delta A_{410 \text{ nm}}^{\text{test}}) / (8800 * \text{time} * \text{leaf extract volume})$

$A_{410 \text{ nm}}^{\text{blank}}$ = Absorbance in the **blank** at 410 nm

$\Delta A_{410 \text{ nm}}^{\text{control}}$ = Absorbance variation in the **control** sample at 410 nm within 120 seconds

$\Delta A_{410 \text{ nm}}^{\text{test}}$ = Absorbance variation in the **test** sample at 410 nm within 120 seconds

8800 = extinction coefficient of *p*-nitroanilide at 410 nm

time = 120 seconds

leaf extract volume = Volume of inhibitor source used (in milliliters)

The presence of trypsin inhibitors in the leaf extract decreases the enzymatic activity and this inhibition can be represented in the Lineweaver-Burk graph, where, as the inhibitor concentration increases, the slope of the line also increases.

Lineweaver-Burk plot analysis of the inhibitory activity of soybean leaf extract toward trypsin. In blue, the kinetics in the absence of inhibitors (**control**). The kinetics in the presence of inhibitors (**test**) at $0.5 K_i$ and K_i are represented in orange and gray, respectively.