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Enzymatic Assay of Trypsin Inhibition

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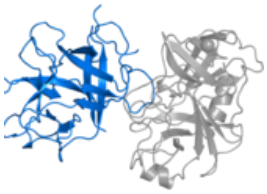

Neilier Junior[1]

[1]University of Manitoba

Neilier Junior
University of Manitoba, Universidade Federal de Viçosa

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

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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

MATERIALS

- Tris
- DMSO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1435
- Calcium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C4904
- Trypsin from bovine pancreas **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8003
- Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (L-BApNA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**B3279

Troubleshooting

Before start

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-BA*p*NA stock solution:
Dilute 130.47 mg L-BA*p*NA in 5 mL DMSO (concentration: 6.0 10^{-2} M). Store at -20 °C and protected from light.

L-BA*p*NA 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 $CaCl_2$. 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 BA*p*NA 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 BA*p*NA 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 BA*p*NA 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**

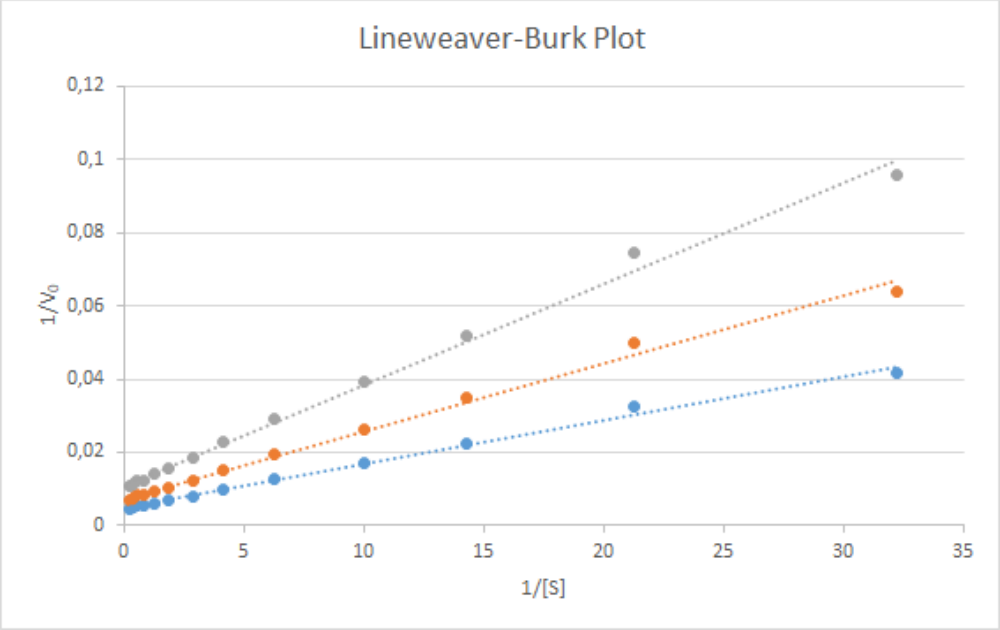
‰ Inhibition = ($\Delta A_{410\ nm}$**control** - $\Delta A_{410\ nm}$**test**) * 100 / ($\Delta A_{410\ nm}$**control** - $A_{410\ nm}$**blank**)

or

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

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