Trypsin Assay Kit

Catalog No.: abx298846

Size: 96 tests

Storage: Store all kit components at -20°C in the dark.

Application: For quantitative detection of Trypsin activity in tissue homogenates.

Detection Range: 1.86 U/L – 100.65 U/L

Sensitivity: 1.86 U/L

Introduction: Trypsin is a serine protease primarily found in the small intestine of many vertebrates. It is cleaved from trypsinogen, an inactive proenzyme synthesized in the pancreas. Active trypsin predominantly cleaves peptide chains at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline. It is used in numerous biotechnological processes. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 405 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 2 × 50 ml
3. Substrate: 2 vials
4. Standard: 2 vials
5. Reconstitution Buffer: 4 ml

Materials Required But Not Provided

1. Microplate reader (405 nm)
2. High-precision pipette and sterile pipette tips
3. PBS (0.01 M, pH 7.4)
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 0.5 ml of Reconstitution Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use, and use within 4 hours of reconstitution.

- **Reaction Working Solution**
  Dilute Substrate Solution with Assay Buffer 25-fold. Mix fully and prepare immediately before use.

- **Stock Standard Solution**
  Add 1 ml of Reconstitution Buffer to the Standard vial and mix thoroughly. The prepared solution may be stored at -20°C for up to 1 week. Avoid repeated freeze-thaw cycles.

- **Standard Solution (1 mmol/L)**
  Dilute Stock Standard Solution with Assay Buffer 20-fold. Mix fully and use within 4 hours of preparation.

2. Sample

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice, then centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately (abx097193).

Recommended dilution factors are as follows. Dilute samples using Assay Buffer.

Sample type	Dilution factor
10% Mouse small intestine tissue homogenate	2-5
10% Mouse large intestine tissue homogenate	2-5
10% Rat intestine tissue homogenate	2-5

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Label 7 tubes with 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1.0 mmol/L. Prepare the tubes as follows:

Volume of 1 mmol/L Standard (µl)	Volume of Assay Buffer (µl)	Standard concentration (mmol/L)
0	200	0
40	160	0.2
60	140	0.3
80	120	0.4
120	80	0.6
140	60	0.7
160	40	0.8
200	0	1.0

2. Set the sample and standard wells and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of prepared standard solutions to the standard wells.
4. Add 10 µl of sample solutions to the sample wells.
5. Add 160 µl of Reaction Working Solution to the standard and sample wells.
6. Tap the plate gently to mix, and measure the absorbance at 405 nm ($\boldsymbol{A_1}$).
7. Incubate at 37°C for 10 minutes accurately.
8. Measure the absorbance at 405 nm ($\boldsymbol{A_2}$).

C. Calculations

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $\boldsymbol{y = ax + b}$. Based on this curve, the concentration of Activity of Trypsin in each sample well can be derived with the following formula:

One unit of Trypsin activity is defined as the amount of enzyme required to produce 1 µmol of p-NA per minute.

Trypsin activity per g of protein:

$$\mathbf{Trypsin\ (U/g)} = \frac{\Delta \boldsymbol{A} - \boldsymbol{b}}{\mathbf{a} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \boldsymbol{f} \times \mathbf{1000}$$

where:

$\Delta \boldsymbol{A}$	$\boldsymbol{A_2 - A_1}$
$\mathbf{OD_{Standard}}$	Absorbance of highest standard
$\mathbf{OD_{Blank}}$	Absorbance of blank (0 mmol/L standard)
$\boldsymbol{y}$	$\mathbf{OD_{Standard} - OD_{Blank}}$
$\boldsymbol{a}$	Gradient of the standard curve
$\boldsymbol{b}$	Intercept of the standard curve
$\mathbf{C_{Protein}}$	Concentration of protein (in g/L)
$\boldsymbol{f}$	Sample dilution factor
$\mathbf{T}$	Reaction time (10 minutes)
$\mathbf{1000}$	1 mmol/L = 1000 µmol/L