QuantiFluo™ Trypsin Inhibitor Assay Kit (DTRI-100)

Fluorimetric Inhibitor Screening Assay for Trypsin

DESCRIPTION

TRYPSIN (EC 3.4.21.4) is a digestive, serine protease that hydrolyzes dietary proteins in many eukaryotic and prokaryotic organisms. Trypsin predominantly cleaves peptide chains at the carboxyl side of lysine and arginine amino acids, but not before proline. BioAssay System's QuantiFluo™ Trypsin Inhibitor Assay Kit uses a fluorescein isothiocyanate (FITC)-labeled synthetic substrate. The fluorescein label is highly quenched. Upon digestion by trypsin present in the sample, the substrate is cleaved into smaller peptides, which abolishes the quenching of the fluorescence label. The fluorescence or fluorescence polarization (FP) of the FITC-labeled fragments is measured at $\lambda_{ex/em}$ = 485/530 nm. Inhibition is determined by the decrease in fluorescence.

KEY FEATURES

Safe. Non-radioactive assay.

Homogeneous and convenient. Homogenous "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and High-throughput. Can be readily automated to assay thousands of samples per day. Robust assay with a Z' factor of > 0.7.

APPLICATIONS

For evaluation of drugs and screening of potential inhibitors to trypsin proteases.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **Substrate:** 100 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

Prior to the assay, equilibrate all components to room temperature. This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature. *Note: This kit does not include enzyme or inhibitor. Customers should provide enzyme or inhibitor of their choice.*

Sample Preparation:

To prevent autodigestion, pure trypsin solutions can be stored long term at -80°C under acidic conditions (1 mM HCl, pH 3.0). Dilute purified trypsin in Assay Buffer. Trypsin should be kept on ice and used as soon as possible. Dissolve test compounds in a solvent of choice (e.g. DMSO). It is prudent to first test the tolerance of the solvent by the enzyme of choice. For porcine trypsin, the final DMSO concentration in the assay should be < 2%.

Inhibitor Screening in 96-well plate:

1. Transfer 30 µL of trypsin into separate wells of a black, flat-bottom 96-well plate. Also, prepare 30 µL of trypsin ("Control") and 30 µL of Assay Buffer ("Blank") wells, respectively.
2. To the Control and Blank wells, add 10 µL of the solvent in buffer that the test compounds are dissolved in. For example, if the test compounds are dissolved in buffer containing 0.1% DMSO, add 10 µL of this solution to these wells.
3. To the remainder of the wells containing enzyme, add 10 µL of the test compounds. Tap plate to mix and incubate for 15 min at room temperature to allow the inhibitor to block Enzyme activity.
4. Prepare enough Working Reagent for all wells by mixing 1 µL Substrate and 99 µL Assay Buffer for each well. Add 80 µL of the Working Reagent to all wells. Briefly tap to mix. Incubate for 30 min at room temperature, protected from light. Read the fluorescence or FP at $\lambda_{ex/em}$ = 485/530 nm.

CALCULATION

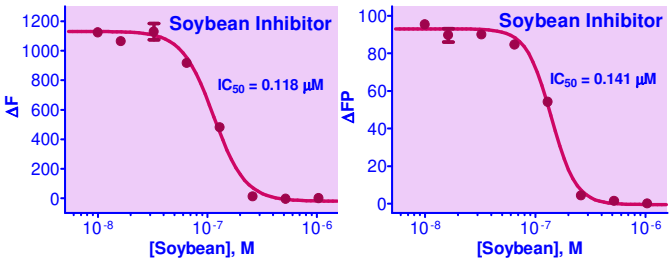
Trypsin inhibition for a test compound is calculated as follows:

$$\text{Enzyme Activity \%} = \frac{(F_{Compound} - F_{Blank})}{(F_{Control} - F_{Blank})} \times 100\%$$

Where $F_{Compound}$, $F_{Control}$, and F_{Blank} are the fluorescence or FP values at $\lambda_{ex/em}$ = 485/530 nm of the test compound, no inhibitor "Control", and the no enzyme "Blank" wells, respectively.

MATERIALS REQUIRED, BUT NOT PROVIDED

Purified trypsin (e.g. Sigma Aldrich, cat# T8003) and, if desired, a control trypsin inhibitor (e.g. Trypsin Soybean Inhibitor, Cayman Chemical, cat# 14502; Phenylmethanesulfonyl fluoride (PMSF), Sigma Aldrich Cat# P7626). Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes, and a plate reader capable of reading fluorescence or fluorescence polarization at $\lambda_{ex/em}$ = 485/530 nm.



Trypsin inhibition curves. *Left:* Trypsin was incubated with various concentrations of trypsin soybean inhibitor to generate a fluorescent IC_{50} curve. *Right:* A second IC_{50} curve was generated for FP measurements.

LITERATURE

1. Manea, M., Mezo, G., Hudecz, F., & Przybylski, M. (2007). Mass spectrometric identification of the trypsin cleavage pathway in lysyl-proline containing oligotuftsin peptides. *Journal of Peptide Science*. 13(4): 227–236.

2. López-Otín, C., Bond, J. S. (2008). Proteases: multifunctional enzymes in life and disease. *Journal of Biological Chemistry*, 283(45): 30433–30437.

3. Shah, D., Mital, K. (2018). The Role of Trypsin: Chymotrypsin in Tissue Repair. *Advances in Therapy*, 35(1): 31–42.