Technical Bulletin

Trypsin Inhibitor Assay Kit

Catalogue Number MAK620

Product 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.

The 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 λex/em = 485/530 nm. Inhibition is determined by the decrease in fluorescence.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK620A
- Substrate 100 µL
  Catalogue Number MAK620B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescent multiwell plate reader capable of λex/em = 485/530.
- Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent) Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Purified trypsin (Catalogue number T8003)
- Control trypsin inhibitor
- Phenylmethanesulfonyl fluoride (PMSF) (Catalogue number P7626)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped at ambient temperature. Store the kit at -20°C upon receipt.

Preparation Instructions

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%.

Procedure

Inhibitor Screening Reaction

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.
5. Read the fluorescence or FP at λex/em = 485/530 nm.

Results

Calculations

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:

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

Figure 1.
Trypsin was incubated with various concentrations of trypsin soybean inhibitor to generate a fluorescent IC50 curve.

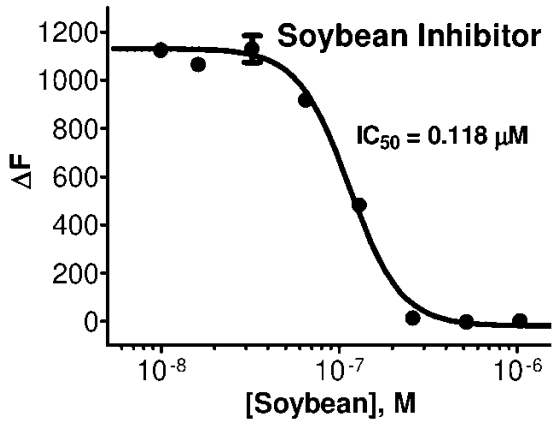
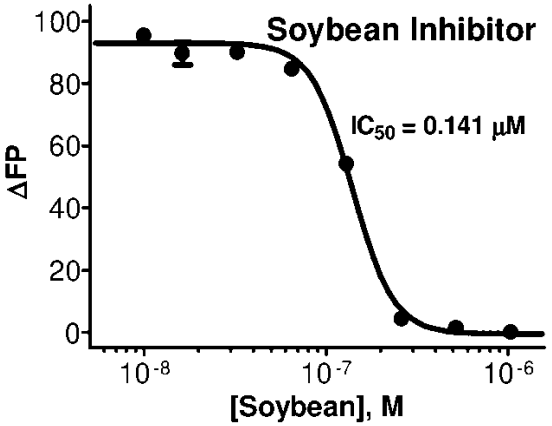


Figure 2.
A second IC50 curve was generated for FP measurements.



References

1. Manea, M., Mezo, G., Hudecz, F., & Przybylski, M. (2007). Mass spectrometric identification of the trypsin cleavage pathway in lysylproline 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.

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