QuantiFluo™ Pepsin Inhibitor Assay Kit (DPEI-200)

Fluorimetric Inhibitor Screening Assay for Pepsin

DESCRIPTION

PEPSIN (EC 3.4.23.1) is a digestive, serine protease that hydrolyzes dietary proteins in many eukaryotic and prokaryotic organisms. Pepsin predominantly cleaves peptide chains at the amino side of the aromatic phenylalanine, tryptophan, and tyrosine amino acids. BioAssay Systems' QuantiFluo™ Pepsin Inhibitor Assay Kit uses a fluorescein isothiocyanate (FITC)-labeled synthetic substrate. The fluorescein label is highly quenched. Upon digestion by pepsin 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 pepsin proteases.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 20 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 must provide enzyme or inhibitor of their choice.*

Sample Preparation:

To reduce speed of autodigestion, pure pepsin solutions can be stored long term at -80°C under acidic conditions (10 mM HCl, pH 2.0). Prior to the assay, prepare enough 10 mM HCl (e.g. 10 mL) for all test wells of the 96-well plate. Dilute purified pepsin in HCl solution. Pepsin 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 pepsin, the final DMSO concentration in the assay should be < 2%. For the purposes of this assay, it is recommended that compounds are dissolved in acidic solvent (e.g. 10 mM HCl, pH 2.0) to provide the best conditions for pepsin enzyme activity.

Inhibitor Screening in 96-well plate:

1. Transfer 30 µL of 27 U/L pepsin (final concentration of 5.4 U/L in reaction) into separate wells of a black, flat-bottom 96-well plate. Also, prepare 30 µL of pepsin ("Control") and 30 µL of 10 mM HCl ("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 10 mM HCl 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 at the desired concentrations. Tap plate to mix and incubate for 15 min at room temperature to allow the inhibitor to block Pepsin activity.
4. Prepare enough Working Reagent for all wells by mixing 0.5 µL Substrate and 12 µL 10 mM HCl for each well. Add 10 µL of the Working Reagent to all wells. Tap plate well to mix. Incubate for 30 min at room temperature, protected from light.
5. Add 100 µL Assay Buffer to all wells to neutralize the fluorescent substrate and read fluorescence or FP at $\lambda_{ex/em}$ = 485/530 nm.

CALCULATION

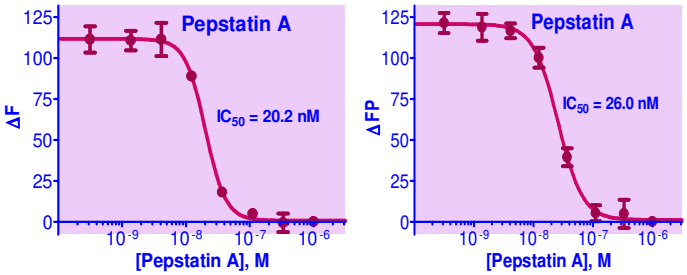
Pepsin inhibition for a test compound is calculated as follows:

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

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

10 mM HCl solution, purified pepsin (e.g. MP Biomedicals, cat# 102598) and, if desired, a control pepsin inhibitor (e.g. Pepstatin A, Cayman Chemical, cat# 9000469). 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.



Pepsin inhibition curves. *Left:* Pepsin was incubated with various concentrations of Pepstatin A to generate a fluorescent IC_{50} curve. *Right:* A second IC_{50} curve was generated using FP measurements.

LITERATURE

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

2. Marciniszyn, J., Jr, Hartsuck, J. A., & Tang, J. (1977). Pepstatin inhibition mechanism. *Advances in experimental medicine and biology*, 95, 199–210.

3. Sancho, J and Campos, L. (2003). The active site of pepsin is formed in the intermediate conformation dominant at mildly acidic pH. *FEBS Letters*, 538(1-3): 89-95.