Urokinase Inhibitor Screening Assay Kit (BA0228)

Product Datasheet | SKU BA0228

Description

A quantitative fluorimetric inhibitor screening assay for urokinase. The fluorimetric substrate reacts with urokinase, and the presence of an inhibitor decreases the fluorescence measured at λex/em = 380/450 nm, allowing potential inhibitors to be evaluated and ranked.

Specifications

Detection Method	Fluorimetric determination at λex/em = 380/450 nm
Detection Range	Inhibitor screening assay for urokinase
Sample Type	Compounds that affect urokinase activity
Species Reactivity	All
Assay Time	30 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped at room temperature. Store all components at -20°C upon receipt.
Shelf Life	12 months after receipt.
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
10 mM Inhibitor	40 µL	-20°C
Substrate	600 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0228	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Equilibrate all components to room temperature and briefly centrifuge tubes before opening. The Working Reagent (WR) should be prepared fresh for each assay run. Note: enzyme is not included; an enzyme of interest is required.
2. Prepare the enzyme in an enzyme buffer, e.g. 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.08% BSA. Experimentally determine the optimal amount of enzyme per well if using an enzyme other than native human urokinase plasminogen activator.
3. Dissolve test compounds in a solvent of choice (e.g. DMSO); first test the tolerance of the solvent by the enzyme. Amiloride hydrochloride may be used to generate a reference IC50 curve.
4. Transfer 5 µL of Enzyme into separate wells of a black, flat-bottom 96-well plate. Reserve one well for the No Inhibitor Control and one for the No Enzyme Blank: add 5 µL Enzyme to the Control and 5 µL Assay Buffer to the Blank.
5. To the Control and Blank wells, add 25 µL of the solvent in Assay Buffer in which the test compounds are dissolved.

6. To the remaining Enzyme-containing wells, add 25 µL of the test compounds. Tap to mix and incubate for 10 min at room temperature to allow the inhibitor to block enzyme activity.
7. Prepare WR by mixing 5 µL Substrate and 95 µL Assay Buffer per well. Transfer 90 µL WR to all wells. Tap to mix and incubate for 30 min at room temperature.
8. Read the fluorescence intensity at λex/em = 380/450 nm.
9. 384-well format: incubate 5 µL Enzyme with 15 µL Test Compounds for 10 min, then add 60 µL WR.

Calculation

Enzyme Activity % = (F_Compound – F_Blank) / (F_Control – F_Blank) × 100%, where F_Compound, F_Control and F_Blank are the fluorescence values of the test compound, Control and Blank wells at 30 min.

Notes

- This assay is based on a kinetic reaction; addition of the Working Reagent to samples should be quick and mixing brief but thorough.
- The assay can be run at room temperature.
- Enzyme is not included in the kit. An enzyme of interest is required for this inhibitor assay.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.