QuantiFluo™ Urokinase Inhibitor Screening Kit (DUKI-100)

Quantitative Fluorimetric Inhibitor Screening Assay for Urokinase

DESCRIPTION

UROKINASE PLASMINOGEN ACTIVATOR (urokinase, uPA) is a key serine protease involved in the degradation of the extracellular matrix that catalyzes the conversion of plasminogen to active plasmin. It acts as a thrombolytic agent to break up blood clots and when over-expressed, has been reported to influence the growth of certain malignant tumors (breast, prostate, etc.). BioAssay Systems' DUKI-100 Kit provides a convenient fluorimetric means to screen for potential urokinase inhibitors. In this assay, the fluorimetric substrate reacts with urokinase and an inhibitor will decrease the fluorescence at $\lambda_{ex/em}$ = 380/450 nm.

KEY FEATURES

Safe. Non-radioactive assay.

Fast. Assay is completed within a 30 minute reaction time**.**

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For evaluation of drugs and screening potential inhibitors of urokinase.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **10 mM Inhibitor:** 40 µL
Substrate: 600 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 12 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

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent (*WR*) to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. The assay can be run at room temperature. *Note: Enzyme is not included in the kit. An enzyme of interest is required for this inhibitor assay.*

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The *WR* should be prepared fresh for each assay run.

Enzyme Preparation: Enzyme should be prepared in an enzyme buffer, e.g. 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.08% BSA. The following protocol is optimized for Native Human Urokinase Plasminogen Activator from Cell Sciences (Cat # CRU000A). If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Test Compound Preparation: 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. In the example below, human urokinase (Cell Sciences, Cat# CRU000A) was found to tolerate up to 0.3% DMSO. If a reference IC_{50} curve is desired, amiloride hydrochloride inhibitor can be prepared as a test compound according to the concentrations found in the example IC_{50} curve.

Inhibitor Screening in 96-Well Plate

1. Transfer 5 µL of Enzyme into separate wells of a black, flat-bottom 96-well plate. Reserve at least one well for No Inhibitor Control ("Control") and one for the No Enzyme Blank ("Blank"): Add 5 µL of Enzyme and 5 µL of Assay Buffer to the Control and Blank wells respectively.
2. To the Control and Blank wells, add 25 µL of the solvent in Assay Buffer that the test compounds are dissolved in. For example, if the test compounds are dissolved in Assay Buffer containing 0.1% DMSO, add 25 µL of this solution to these wells.
3. To the remainder of the wells containing Enzyme, add 25 µL of the test compounds. Tap plate to mix and incubate for 10 min at RT to allow the inhibitor to block Enzyme activity.
4. Prepare enough *WR* for all wells by mixing 5 µL of Substrate and 95 µL of Assay Buffer for each well. Transfer 90 µL of *WR* to all wells. Briefly tap plate to mix. Incubate for 30 min at room temperature.
5. Read the fluorescence intensity at $\lambda_{ex/em}$ = 380/450 nm.

CALCULATION

The percent of Urokinase activity in the presence of test compounds is calculated as follows:

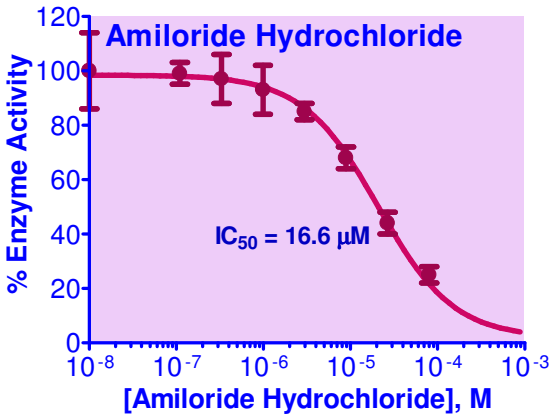
$$\text{Enzyme Activity \%} = \frac{(F_{Compoupd} - F_{Blank})}{(F_{Control} - F_{Blank})} \times 100\%$$

Where $F_{Compound.}$, $F_{Control}$ and F_{Blank} are the fluorescence values of the test compound, Control, and Blank wells at 30 min.

Inhibitor Screening in 384-Well Plate: The procedure is essentially the same as for the 96-well plate assay, except that 5 µL Enzyme and 15 µL of Test Compounds are incubated for 10 min, then 60 µL *WR* is added.

MATERIALS REQUIRED, BUT NOT PROVIDED

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.



Inhibition of human urokinase activity by amiloride. Assays are performed in duplicate in 96-well plate according to the standard protocol. Enzyme was incubated with amiloride in the presence of 0.3% DMSO.

LITERATURE

1. Law, B. et al. (2004). Design, Synthesis, and Characterization of Urokinase Plasminogen-Activator-Sensitive Near-Infrared Reporter. Chemistry & Biology. 11, 99–106.

2. Mahmood N, Mihalcioiu C and Rabbani SA (2018) Multifaceted Role of the Urokinase-Type Plasminogen Activator (uPA) and Its Receptor (uPAR): Diagnostic, Prognostic, and Therapeutic Applications. Front. Oncol. 8, 24.

3. Rockway TW, Nienaber V, Giranda VL. (2002). Inhibitors of the protease domain of urokinase-type plasminogen activator. Curr Pharm Des. 8(28), 2541-58.