



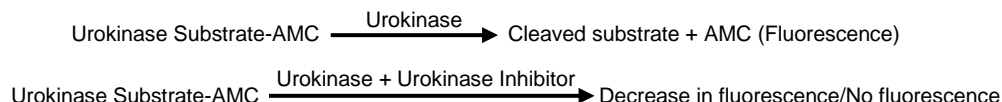
## Urokinase Inhibitor Screening Kit (Fluorometric)

rev. 6/14

(Catalog # K727-100; 100 assays; Store kit at -20°C)

### I. Introduction:

Urokinase (Urokinase-type plasminogen activator, uPA, EC 3.4.21.73) is a serine protease, which catalyzes the conversion of the plasminogen to active plasmin, which in turn participates in thrombolysis. Urokinase inhibitors have potential applications in cancer therapy. Biovision's Urokinase Inhibitor Screening Kit utilizes the ability of Urokinase to cleave a synthetic AMC-based peptide substrate to release AMC, which can be detected by measuring its fluorescence at Ex/Em = 350/450 nm. In the presence of the Urokinase specific inhibitors, the enzyme activity is inhibited or completely abolished. This assay kit is a simple, sensitive, and rapid tool to screen potential Urokinase inhibitors.



### II. Application:

- Screening potential inhibitors of Urokinase
- Characterize/study Urokinase inhibitors

### III. Kit Contents:

Components	K727-100	Cap Code	Part Number
Urokinase Assay Buffer	25 ml	NM	K727-100-1
Urokinase Substrate	0.20 ml	Red	K727-100-2
Human Urokinase	1 vial	Green	K727-100-3
Urokinase Inhibitor [GGACK Dihydrochloride] (10 mM)	10 µl	Blue	K727-100-4

### IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer (ELISA reader)

### V. Storage and Handling:

Store kit at -20°C, protected from light. Warm Urokinase Assay Buffer and Substrate to room temperature before use. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

### VI. Reagent Preparation and Storage Conditions:

**Human Urokinase:** Reconstitute with 1.1 ml of Urokinase Assay Buffer to prepare the stock solution (100 IU/ml). Aliquot & store at -80°C. Avoid repeated freeze/thaw. Use within two months.

### VII. Urokinase Inhibitor Screening Protocol:

**1. Urokinase Enzyme Solution Preparation:** For each well, prepare 50 µl of Urokinase enzyme solution.

40 µl Urokinase Assay Buffer  
10 µl Human Urokinase stock solution

Mix well and add 50 µl/well into a 96-well microtiter plate.

**2. Screening compounds, Inhibitor Control & Blank Control preparations:** Dissolve test inhibitors into appropriate solvent. Add 1 µl of 100x solution of the test inhibitor, Urokinase Assay Buffer or Urokinase Inhibitor into wells containing Urokinase enzyme solution as sample screen, Enzyme Control (EC) or Inhibitor Control (IC). Incubate at room temperature for 10-15 min.

**3. Urokinase Substrate Preparation:** For each well, prepare 49 µl of Urokinase Substrate solution.

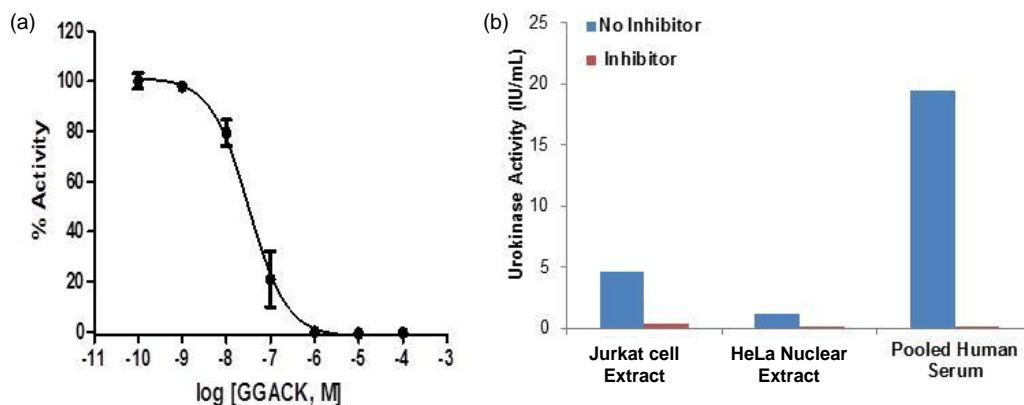
47 µl Urokinase Assay Buffer  
2 µl Urokinase Substrate

Mix & add 49 µl of Urokinase Substrate solution into each well. Mix well.

**4. Measurement:** Measure the fluorescence in a kinetic mode for 30-60 min (Ex/Em = 350/450 nm). Choose two time points ( $T_1$  &  $T_2$ ) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU<sub>1</sub> and RFU<sub>2</sub>).

**5. Calculations:** Calculate the slope for all test Inhibitor Samples [S] & Enzyme Control (EC) by dividing the net  $\Delta$ RFU (RFU<sub>2</sub>-RFU<sub>1</sub>) values with the time  $\Delta T$  ( $T_2-T_1$ ).

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of EC} - \text{Slope of Sample}}{\text{Slope of EC}} \times 100$$



**Figure:** Inhibition of Urokinase activity by the Urokinase Inhibitor [GGACK Dihydrochloride] (Cat. # 1847) (a). Urokinase activity of Jurkat cell extract [10  $\mu$ l (Cat. # 1106)], HeLa nuclear extract [10  $\mu$ l (Cat. # 1641)] and Pooled human serum (10  $\mu$ l) was measured in the presence and absence of the Urokinase Inhibitor (10<sup>-4</sup> M) (b). Assays were performed following the kit protocol.

#### VIII. RELATED PRODUCTS:

Urokinase Activity Fluorometric Assay Kit  
Human Calpain 1 Inhibitor Screening Kit (Fluorometric)  
HDAC Inhibitor Drug Screening Kit  
HDAC-8 Inhibitor Drug Screening Kit  
Granzyme B Inhibitor Screening Kit  
MMP-3 Inhibitor Screening Kit  
CETP inhibitor Drug Screening Kit (Fluorometric)  
Protease Inhibitor Cocktail  
EZBlock™ Protease Inhibitor Cocktail, EDTA-Free

Enteropeptidase Inhibitor Screening Assay Kit (Fluorometric)  
DPP4 Inhibitor Screening Kit (Fluorometric)  
HDAC-3 Inhibitor Drug Screening Kit  
Granzyme B Activity Fluorometric Assay Kit  
MMP-1 Inhibitor Screening Kit  
TACE inhibitor Screening Assay Kit (Fluorometric)  
PLTP inhibitor Drug Screening Kit (Fluorometric)  
EZBlock™ Protease Inhibitor Cocktail  
Enteropeptidase Activity Assay Kit (Fluorometric)

**FOR RESEARCH USE ONLY! Not to be used on humans.**