ab283409 – Urokinase Inhibitor Screening Kit (Fluorometric)

For the screening of potential Urokinase inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283409

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Urokinase Assay Buffer	25 mL	-20°C
Urokinase Substrate	200 µl	-20°C
Human Urokinase	1 vial	-20°C
Urokinase Inhibitor	10 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Urokinase Assay Buffer: Bring the Urokinase Assay Buffer to room temperature (RT) before use.

Urokinase Substrate: Bring the Urokinase Substrate to RT before use.

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

Assay Protocol

Urokinase Enzyme Solution Preparation:

1. Mix enough solution for the number of assays to be performed. For each well, prepare 50 µl Urokinase Enzyme Solution containing:

	Volume
Urokinase Assay Buffer	40 µl
Human Urokinase Stock Solution	10 µl

2. Mix and add 50 µl of solution into each well. Mix well with gentle shaking.

Screening Compounds, Inhibitor Control, and Blank Control Preparation:

1. Dissolve test inhibitors into appropriate solvent.
2. 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).
3. Incubate at room temperature for 10-15 minutes.

Version 1, Last updated June 14, 2021

Urokinase Substrate Preparation:

1. Mix enough solution for the number of assays to be performed. For each well, prepare 49 µl Urokinase Enzyme Solution containing:

	Volume
Urokinase Assay Buffer	47 µl
Urokinase Substrate	2 µl

2. Mix and add 49 µl of solution into each well. Mix well with gentle shaking.

Measurement

1. Measure the fluorescence in a kinetic mode for 30-60 minutes (Ex/Em = 350/450 nm).
2. Choose two time points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

Calculations:

1. Calculate the slope for all test Inhibitor Samples [S] and Enzyme Control (EC) by dividing the net ΔRFU (RFU2-RFU1) values with the time ΔT (T2-T1).

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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)