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BioVision
BioVision Incorporated

Urokinase Activity Fluorometric Assay Kit

rev. 10/13

(Catalog # K728-100; 100 assays; Store kit at -20˚C)

I. Introduction:

Urokinase (urokinase-type plasminogen activator or 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. Biovision's Urokinase Fluorimetric Activity Assay Kit utilizes an AMC-based peptide substrate containing the recognition sequence for Urokinase. Urokinase present in the sample catalyzes the cleavage of the substrate and releases AMC, which can be easily quantified by measuring its fluorescence at Ex/Em = 350/450 nm. This sensitive assay detects very low levels of Urokinase activity in biological samples (as low as 0.02 IU/ml).

$$\text{Substrate-AMC} \xrightarrow{\text{Urokinase}} \text{Cleaved Substrate + AMC (Fluorescence)}$$

II. Application:

- Measuring Urokinase activity of purified enzymes
- Measuring Urokinase activity in serum & cell/nuclear extracts

III. Sample Type:

- Cell & nuclear extract
- Serum

IV. Kit Contents:

Components	K728-100	Cap Code	Part Number
Urokinase Assay Buffer	25 ml	NM	K728-100-1
Cell Lysis Buffer	25 ml	WM	K728-100-2
Urokinase Substrate	0.20 ml	Red	K728-100-3
Human Urokinase (Standard/Positive Control)	1 vial	Green	K728-100-4

V. User Supplied Reagents and Equipment:

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

VI. 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.

VII. Reagent Preparation and Storage Conditions:

- **Human Urokinase (Standard/Positive Control):** Reconstitute with 1.1 ml of Urokinase Assay Buffer to prepare Urokinase stock solution of 100 IU/ml. Mix well, do not vortex. Aliquot and store at -80˚C. Avoid repeated freeze/thaw. Use within two months.

VIII. Urokinase Activity Assay Protocol:

1. **Sample Preparation:** Serum samples can be diluted with Urokinase Assay Buffer & measured directly. Rapidly lyse cells with 4X cell volume of Cell Lysis Buffer. Incubate on ice for 5 min. Centrifuge at max. speed for 10 min. at 4˚C. Collect the supernatant. Add 1-50 µl sample per well and bring the volume to 50 µl with Cell Lysis Buffer. For preparing nuclear extract, use BioVision's Nuclear/Cytosol Fractionation Kit (catalog # K266) or other equivalent method.

   Note: For samples having high fluorescence background, prepare parallel sample well(s) as the sample background control.

2. **Urokinase Standard Curve:**

- **For High Urokinase Activity**: Add 0, 1, 2, 3, 4, and 5 µl of Urokinase stock solution (100 IU/ml) into a series of wells in 96-well plate & adjust the volume to 50 µl with Urokinase Assay Buffer.
- **For Low Urokinase Activity:** Dilute the Urokinase stock solution (100 IU/ml) to 10 IU/ml by adding 10 µl of 100 IU/ml Urokinase stock solution to 90 µl of Urokinase Assay Buffer. Add 0, 1, 2, 3, 4, and 5 µl of the diluted Urokinase solution (10 IU/ml) into a series of wells in 96-well plate & adjust the volume to 50 µl with Urokinase Assay Buffer.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays (Standards & test samples) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Urokinase Assay Buffer	48 µl
Urokinase Substrate	2 µl

   Mix & add 50 µl Reaction Mix to Standard & sample wells. Mix well.

4. **Measurement**: Immediately measure the fluorescence (RFU_1) at Ex/Em = 350/450 nm using a fluorescence microplate reader. Incubate for 30-60 min at room temperature and measure fluorescence again (RFU_2).

   Note: Incubation time depends on the Urokinase activity in the samples. Longer incubation times may be required to generate sufficient fluorescence signal for samples having very low activity.

5. Calculation: Subtract the reading at time zero ($\Delta RFU = RFU_2 - RFU_1$) from all Samples ($\Delta RFU_S$) and Urokinase Standards (ΔRFU_{UK}). Obtain the corrected fluorescence readings for Urokinase Standards by subtracting the 0 Standard reading from all Standards ($\Delta RFU_{UK} - \Delta RFU_{0Standard}$). Plot the Urokinase activity Standard Curve. Calculate the corrected fluorescence for all the test samples ($\Delta RFU_S - \Delta RFU_{0Standard}$). Using the corrected fluorescence reading of test samples and the equation obtained from Urokinase Standards Curve, calculate the activity (IU/ml) of the test samples.

Note: If the sample background control reading is significant, subtract the sample background control fluorescence from that of corrected sample reading.

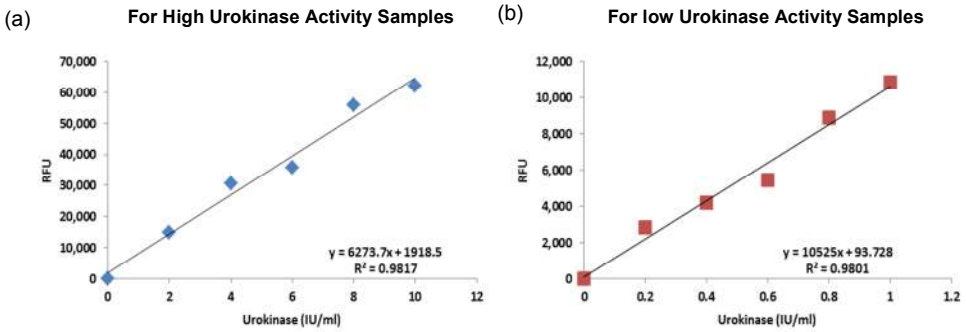

Figure 1: Urokinase Activity Standard Curves, (a) for high Urokinase activity samples and (b) for low Urokinase activity samples. Assays were performed following the kit protocol.

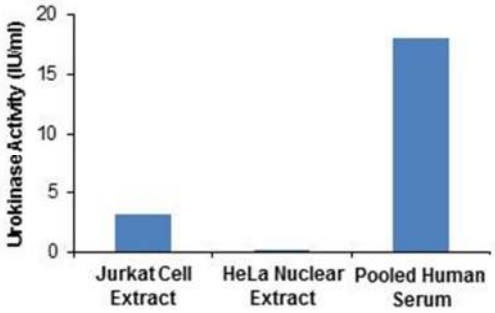

Figure 2: Urokinase activities of Jurkat cell extract [10 µl (Cat. # 1106)], Hela nuclear extract [10 µl (Cat. # 1641)] and Pooled human serum (10 µl). Assays were performed following the kit protocol.

VI. RELATED PRODUCTS:

Urokinase Inhibitor Screening Kit (Fluorometric)
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)

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