QuantiFluo™ Urokinase Assay Kit (DUKN-100)

Quantitative Fluorimetric Assay for Urokinase Activity

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' DUKN-100 Kit provides a convenient fluorimetric method to measure urokinase activity in biological samples. In this assay, the fluorimetric substrate reacts with urokinase so that the increase in fluorescence at $\lambda_{ex/em}$ = 380/450 nm is directly proportional to enzyme activity.

KEY FEATURES

Safe. Non-radioactive assay.

Fast. Assay is completed within a 15 minute reaction time.

Sensitive and accurate. Linear detection range 0.04 - 30 U/L urokinase in a 96-well plate assay.

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

APPLICATIONS

For quantitative determination of urokinase activity determination in biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **2.5 mM Standard:** 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.

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.

Sample Preparation:

Serum can be assay directly.

Urine should be diluted 2-fold or higher in water prior to the assay run.

Standard Preparation: Prepare a 50 µM Premix by combining 5 µL 2.5 mM Standard and 245 µL Assay Buffer. Dilute standards in separate wells of a black flat-bottom 96-well plate as follows.

No.	50 µM Premix + Assay Buffer	Total Volume (µL)	Std (µM)
1	100 µL + 0 µL	100 µL	50 µM
2	60 µL + 40 µL	100 µL	30 µM
3	30 µL + 70 µL	100 µL	15 µM
4	0 µL + 100 µL	100 µL	0 µM

Reaction Preparation:

1. Transfer 10 µL of each sample to separate wells of the plate.

2. Prepare enough *WR* for all sample wells by mixing 5 µL of Substrate and 90 µL of Assay Buffer for each well.

3. Add 90 µL *WR* to all sample wells. Tap plate to mix and incubate for 15 min. Measure fluorescence intensity at $\lambda_{ex/em}$ = 380/450 nm.

CALCULATION

Subtract the blank value (Standard #4) from the standard values and plot ΔF against the standard concentrations. Determine the slope (μM^{-1}) and calculate the urokinase activity in each Sample as follows,

$$\text{Urokinase Activity} = \frac{(F_{Sample} - F_{Blank})}{\text{Slope } (\mu M^{-1})} \times \frac{\text{Reaction Vol } (\mu L)}{t\,(\text{min}) \times \text{Enzyme Vol } (\mu L)} \times n \text{ (U/L)}$$

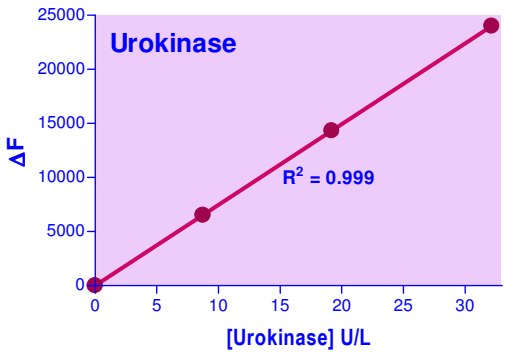
Where F_{Sample} and F_{Blank} are the measured fluorescence values of the sample and blank, t is the reaction time (15 min), Reaction Vol and Enzyme Vol are the reaction (100 µL) and sample (10 µL) volumes, and n is the sample dilution factor.

Unit definition: 1 Unit (U) of urokinase will catalyze the conversion of 1 µmole of the Substrate per min at room temperature and pH 7.4.

Note: If sample urokinase activity exceeds 30 U/L, dilute samples in enzyme buffer and repeat the assay.

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.



96-well Fluorimetric Urokinase Assay

A human serum sample and a human urine sample were assayed for urokinase activity. The baseline levels were 0.085 U/L and 0.012 U/L, respectively.

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. Mazar, A. (2008). Urokinase Plasminogen Activator Receptor Choreographs Multiple Ligand Interactions: Implications for Tumor Progression and Therapy. Clin Cancer Res. 14 (18), 5649 –5655.