

## 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  $\lambda_{\text{ex/em}} = 380/450$  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  $\mu$ L

**Substrate:** 600  $\mu$ L

**Storage conditions.** The kit is shipped at room temperature. Store all components at  $-20^{\circ}\text{C}$  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  $\mu$ M Premix by combining 5  $\mu$ L 2.5 mM Standard and 245  $\mu$ L Assay Buffer. Dilute standards in separate wells of a black flat-bottom 96-well plate as follows.

No.	50 $\mu$ M Premix + Assay Buffer	Total Volume ( $\mu$ L)	Std ( $\mu$ M)
1	100 $\mu$ L + 0 $\mu$ L	100 $\mu$ L	50 $\mu$ M
2	60 $\mu$ L + 40 $\mu$ L	100 $\mu$ L	30 $\mu$ M
3	30 $\mu$ L + 70 $\mu$ L	100 $\mu$ L	15 $\mu$ M
4	0 $\mu$ L + 100 $\mu$ L	100 $\mu$ L	0 $\mu$ M

#### Reaction Preparation:

1. Transfer 10  $\mu$ L of each sample to separate wells of the plate.
2. Prepare enough WR for all sample wells by mixing 5  $\mu$ L of Substrate and 90  $\mu$ L of Assay Buffer for each well.
3. Add 90  $\mu$ L WR to all sample wells. Tap plate to mix and incubate for 15 min. Measure fluorescence intensity at  $\lambda_{\text{ex/em}} = 380/450$  nm.

#### CALCULATION

Subtract the blank value (Standard #4) from the standard values and plot  $\Delta F$  against the standard concentrations. Determine the slope ( $\mu\text{M}^{-1}$ ) and calculate the urokinase activity in each Sample as follows,

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

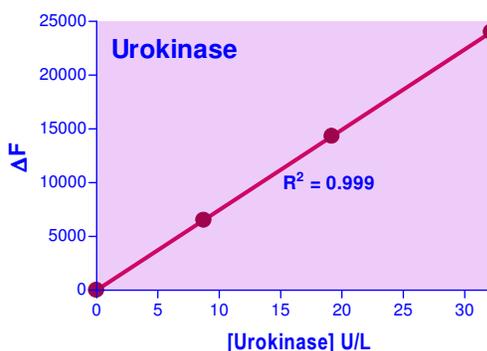
Where  $F_{\text{Sample}}$  and  $F_{\text{Blank}}$  are the measured fluorescence values of the sample and blank,  $t$  is the reaction time (15 min), Reaction Vol and Enzyme Vol are the reaction (100  $\mu$ L) and sample (10  $\mu$ L) volumes, and  $n$  is the sample dilution factor.

**Unit definition:** 1 Unit (U) of urokinase will catalyze the conversion of 1  $\mu$ mole 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, Mihalciou 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.

