Urokinase Assay Kit (BA0229)

Product Datasheet | SKU BA0229

Description

A quantitative fluorimetric assay for urokinase activity. The fluorimetric substrate reacts with urokinase so that the increase in fluorescence at λex/em = 380/450 nm is directly proportional to enzyme activity, with a linear detection range of 0.04 - 30 U/L urokinase in a 96-well plate assay.

Specifications

Detection Method	Fluorimetric determination at λex/em = 380/450 nm
Detection Range	0.04 - 30 U/L urokinase in a 96-well plate assay
Sample Type	Biological samples (e.g. urine and serum)
Species Reactivity	All
Assay Time	15 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped at room temperature. Store all components at -20°C upon receipt.
Shelf Life	12 months after receipt.
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
2.5 mM Standard	40 µL	-20°C
Substrate	600 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0229	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Equilibrate all components to room temperature and briefly centrifuge tubes before opening. The Working Reagent (WR) should be prepared fresh for each assay run.
2. Prepare the enzyme in an enzyme buffer, e.g. 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.08% BSA, when required.
3. Sample preparation: serum can be assayed directly; urine should be diluted 2-fold or higher in water prior to the assay run.
4. Standard preparation: prepare a 50 µM Premix by combining 5 µL 2.5 mM Standard and 245 µL Assay Buffer, then dilute standards in a black flat-bottom 96-well plate to 50, 30, 15 and 0 µM as per the table.
5. Transfer 10 µL of each sample to separate wells of the plate.
6. Prepare enough WR for all sample wells by mixing 5 µL Substrate and 90 µL Assay Buffer per well.
7. Add 90 µL WR to all sample wells. Tap plate to mix and incubate for 15 min. Measure fluorescence intensity at λex/em = 380/450 nm.

Standard Preparation

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

Calculation

Subtract the blank value (Standard #4) from the standard values and plot ΔF against the standard concentrations to determine the slope (μM^{-1}). Urokinase Activity = [(F_Sample – F_Blank) / Slope] × [Reaction Vol / (t × Enzyme Vol)] × n (U/L), where t is the reaction time (15 min), Reaction Vol is 100 µL, Enzyme Vol is 10 µL and n is the sample dilution factor. Unit definition: 1 U of urokinase catalyses the conversion of 1 µmole of Substrate per min at room temperature and pH 7.4. If sample activity exceeds 30 U/L, dilute samples in enzyme buffer and repeat the assay.

Notes

- This assay is based on a kinetic reaction; addition of the Working Reagent to samples should be quick and mixing brief but thorough.
- The assay can be run at room temperature.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.