Urokinase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6845

Detection and Quantification of Urokinase (UK) Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Urokinase (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.

Urokinase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring urokinase activity in a variety of samples. The assay is based on the cleavage of 4 -nitrophenol from the synthetic substrate. Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mL x 4	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer	10 mL x 1	4 °C
Dye Reagent	10 mL x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL Reaction Buffer to generate 10 mmol/ L of standard stock solution , store at 4°C for 1 day or -20°C for3days after reconstitution. Then dilute to 0.3 mmol/L standard top solution by adding 30 µL stock solution into 970 µL distilled water. Per form 2-fold serial dilutions of the top standard solution using Reaction Buffer to make the standard curve. The concentration of standard curve could be 300/150/75/37.5/18. 8/9.4/4.7µmol/L.

Substrate: Add 8 mL Reaction Buffer to dissolve before use. Keep in dark and store at 4°C for 3-4 days or -20°C for 1 -2months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 mL Assay Buffer to dissolve before use, mix. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL Assay Buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s ,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 8000 g 4°C for 10 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquidsamples Detect directly.

6. Assay Procedure

Warm all regents to room temperature before use . Add following reagents in to the microplate :

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	20 µl	--	--	--	--
Standard	--	--	100 µl	--	--
Positive Control	--	--	--	--	20 µl
Distilled water	--	20 µl	--	100 µl	--
Substrate	80 µl	80 µl	--	--	80 µl
Mix, put it in the oven, 37°C for2hours.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, record absorbance measured at 405 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

One unit of UK activity is defined as the enzyme generates 1μmol of p-nitrophenol per minute .

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T} \ (U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \ (U/g)$$

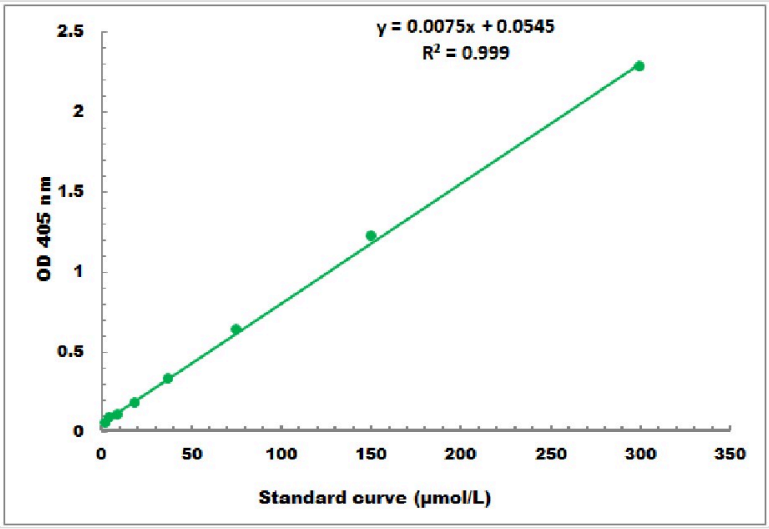
2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

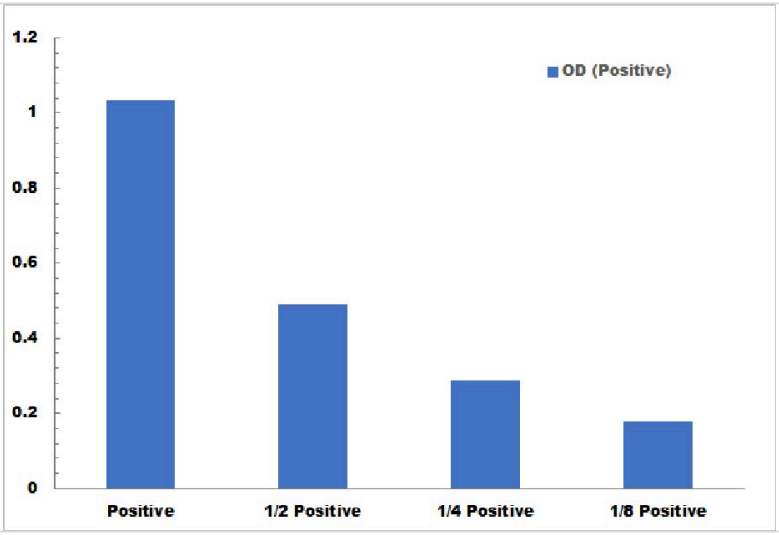
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , minute

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 3μmol/L -300μmol/L



Positive Control reaction in 96 -well plate assay with decreasing the concentration