Urokinase Microplate Assay Kit

User Manual

Catalog # CAK1264

(Version 1.1A)

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.

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I. 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 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.

II. 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	

Note:

Substrate: add 8 ml Reaction Buffer to dissolve before use.

Standard: add 1 ml Reaction Buffer to dissolve before use, then add 30 µl into 970 µl Reaction Buffer, mix; the concentration will be 300 µmol/L.

Positive Control: add 0.1 ml Assay Buffer to dissolve before use, mix.

III. 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

IV. 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 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into 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 for 2 hours.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, record absorbance measured at 405 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$$= 0.0125 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.0125 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 0.0125 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of standard, 300 μmol/L = 0.3 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of standard, 0.1 ml;

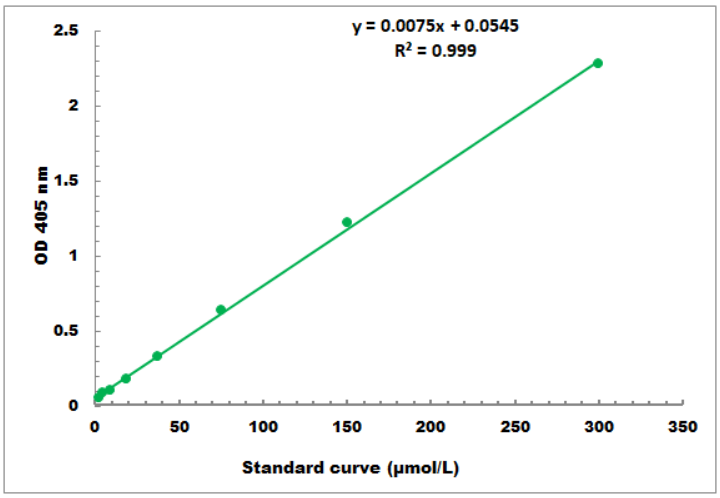
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

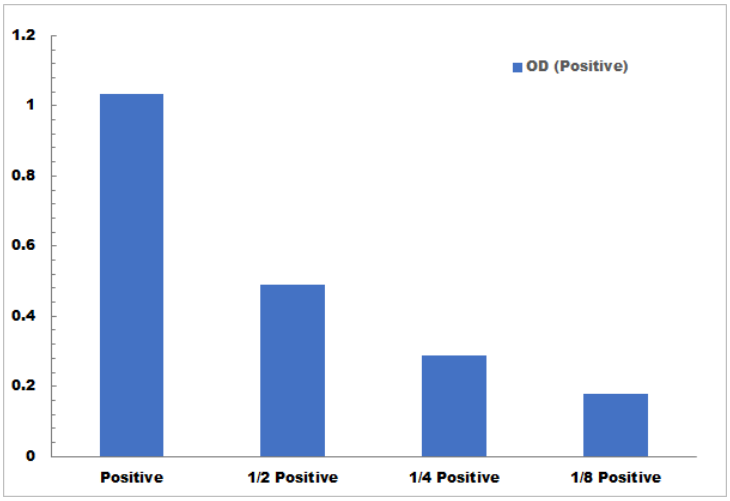
T: the reaction time, 2 hours = 120 minutes.

VII. TYPICAL DATA

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



Detection Range: 3 μmol/L - 300 μmol/L



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES