uPA Activity
Assay Kit

Cat. No. ECM600

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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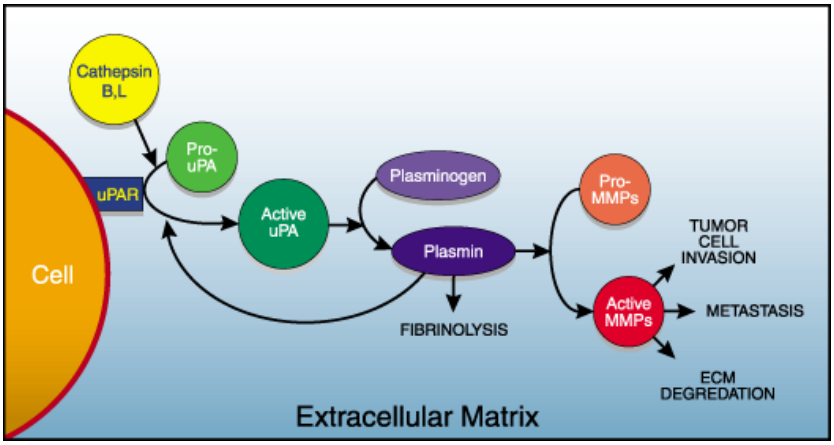
Introduction

Urokinase-type Plasminogen Activator (uPA) is a 52 kDa serine protease which has been implicated in a number of physiological and pathological processes, including tissue remodeling[1], angiogenesis[2], fibrinolysis and tumor spread. When bound to its cell surface receptor, uPA is converted from the single chain pro-form uPA to the active 2-chain HMW-uPA. uPA has been shown to play a role in basement membrane degradation, via a cascade involving activation of plasminogen and the matrix metalloproteinases[3]. Inhibitors of uPA have been shown to slow primary tumor growth and metastasis[4-7].

The CHEMICON uPA Activity Assay Kit provides a quick, efficient and sensitive system for evaluation of uPA activity and for screening of uPA inhibitors. The assay is colorimetric and does not require radioactivity or fluorescence equipment. The assay is sensitive over a range of 0.05-50 units of uPA activity.



Test Principle

The CHEMICON uPA Activity Assay Kit utilizes a chromogenic substrate, which is cleaved by active uPA. Addition of this substrate to a uPA-containing sample results in a colored product, detectable by its Optical Density at 405nm (OD405).

Application

The CHEMICON uPA Assay Kit is ideal for measurement of uPA activity in purified preparations and cell culture, as well as in serum where pathological conditions such as sepsis exist. The assay is also useful for screening inhibitors of the enzymatic activity of uPA.

Each CHEMICON uPA Activity Assay Kit contains sufficient reagents for the evaluation of 96 samples, including uPA from human urine as a positive control. Duplicate or triplicate samples are suggested.

The CHEMICON uPA Activity Assay Kit is intended for research use only; not for diagnostic or therapeutic applications.

Kit Components

1. uPA Positive Control: (Part No. 90058) One lyophilized vial, 1000 units, of uPA from human urine.
2. Chromogenic Substrate: (Part No. 90057) One 5 mg bottle of Tripeptide with pNA group.
3. Assay Buffer, 10X: (Part No. 90091) One 5 mL bottle.

Materials Not Supplied

1. Single or Multichannel Pipette and disposable tips
2. Microplate reader (405 nm)
3. 37°C incubator
4. Clean 96-well microplate for performing incubations.

Assay Instructions

1. Rehydrate uPA positive control with 1 mL deionized H_2O. Following rehydration, aliquot and freeze at –70°C.
2. To each well of a clean 96-well plate, add 10-160 µL of uPA-containing sample or positive control to each well, depending on expected uPA levels.
3. Add sufficient deionized water to bring total volume to 160 µL.
4. Add 20 µL of the Assay Buffer to each well.
5. Rehydrate the Chromogenic Substrate with 2 mL deionized H_2O. Store reconstituted substrate at 2-8°C.
6. Add 20 µL of the Chromogenic Substrate to each well.
7. Incubate at 37°C for 10 minutes-24 hours. Note: Longer incubation is required for samples with low uPA levels.
8. Read Absorbance on a standard microplate reader at 405 nm.

Standard Curve

The following table shows volumes to be used to make a standard curve with the enclosed uPA positive control. This table should be used as a reference only.

uPA Units	uPA Standard (µL)	Deionized H_2O ((µL)	Assay Buffer ((µL)	Substrate ((µL)	Total Volume ((µL)
40	40	120	20	20	200
20	20	140	20	20	200
10	10	150	20	20	200
5.0	5.0	155	20	20	200
2.5	2.5	157.5	20	20	200
1.25	1.25	158.75	20	20	200
0.625	0.625	159.375	20	20	200
0.0	0.0	160	20	20	200

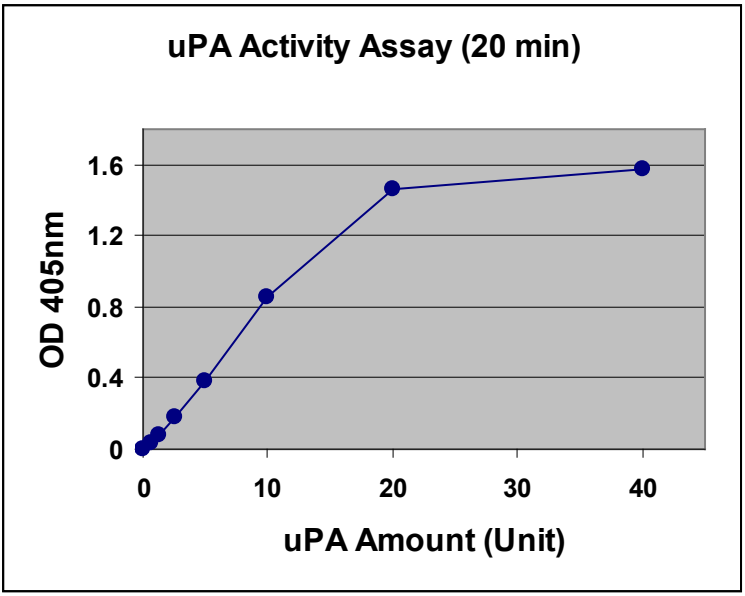
Storage

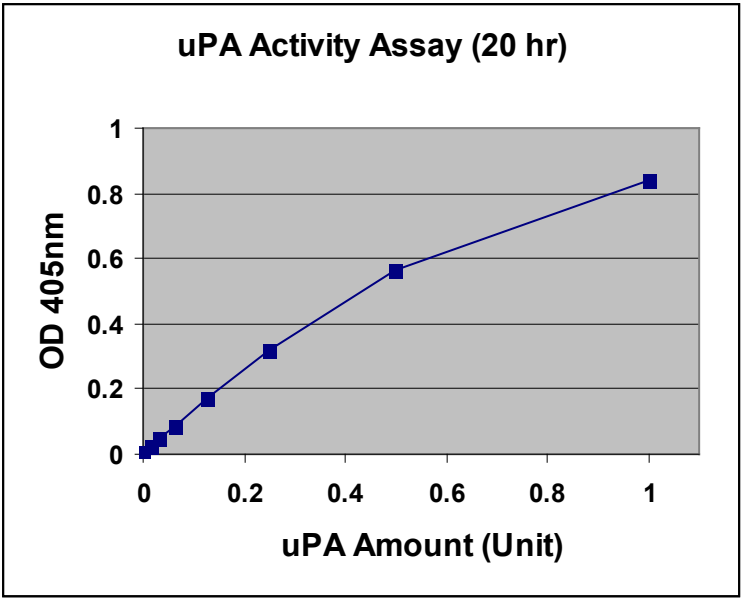
Store kit materials at -20°C for up to 6 months. After reconstitution, store uPA positive control at -70°C and substrate at 2 - 8°C.

Calculation of results

Optical Density values obtained with the CHEMICON uPA Activity Assay Kit may be compared with known standards or other test samples to obtain relative activities. uPA Control provided with the kit is useful as a positive control for quantitative purposes.

The following charts illustrate typical results upon dilution of the uPA Positive Control contained in the kit. One should use the data below for reference only. This data should not be used to interpret actual assay results.





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