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Human uPA Activity ELISA Kit

Catalog # HUPAKT

Strip well format. Reagents for up to 96 tests.

Rev: March 2014

INTENDED USE

Human uPA activity assay is intended for the quantitative determination of active urokinase plasminogen activator in human plasma and urine. **For research use only.**

BACKGROUND

Urokinase plasminogen activator, along with its receptor uPAR, initiates a proteolytic cascade that results in the conversion of plasminogen to plasmin [1]. Clinical studies have indicated that high uPA levels may elevate the risk for tumor invasion and metastasis [2,4]. Increased expression and activity can exert potent arthritogenic properties in rheumatoid arthritis patients [3]. Increased uPA activity may be an implication for the pathophysiology of endometriosis [5].

ASSAY PRINCIPLE

Functionally active uPA present in samples will form a covalent complex with the biotinylated human PAI-1 which is bound to the avidin on the plate. Inactive or complexed uPA will not bind to the plate and will not be detected. Unbound uPA samples are washed away and an anti-uPA primary antibody is added. Excess primary antibody is washed away and bound antibody is then reacted with the horseradish peroxidase secondary antibody. Following an additional washing step, TMB is then used for color development at 450nm. The amount of color development is directly proportional to the concentration of active uPA in the sample.

STANDARD CALIBRATION

The uPA activity standard provided is calibrated against the WHO 2nd International Standard for High Molecular Weight Urokinase distributed by NIBSC (11/184), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 1113L: 500 ng = 56.2 IU

REAGENTS PROVIDED

- **96-well avidin coated microtiter strip plate** (removable wells 8x12) containing avidin, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Biotinylated human PAI-1:** 1 vial lyophilized protein
- **Human uPA activity standard:** 1 vial lyophilized standard
- **Anti-human uPA primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate [6]. This insures that the fast-acting inhibitor for high molecular weight uPA, which is usually present in large excess, is inhibited from quenching uPA activity [5]. Immediately after collection of blood, samples must be centrifuged at 2500Xg for 15 minutes. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The uPA activity samples are stable for up to 5 hours on ice, up to one month frozen at –20°C, or up to 5 months at -70°C.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Biotinylated Human PAI-1 Addition

Add 10ml blocking buffer directly to the biotinylated human PAI-1 vial and agitate gently to completely dissolve contents. Remove microtiter plate from bag and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 500ng/ml standard solution.

Dilution table for preparation of human uPA activity standard:

uPA concentration (ng/ml)	Dilutions
50	900µl (BB) + 100µl (from vial)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Add 100µl uPA activity standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures active uPA in the 0.1-50 ng/ml range. If the unknown is thought to have high uPA levels, dilutions may be made in blocking buffer.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

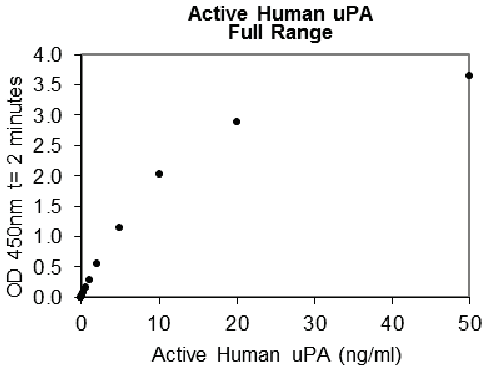
Measurement

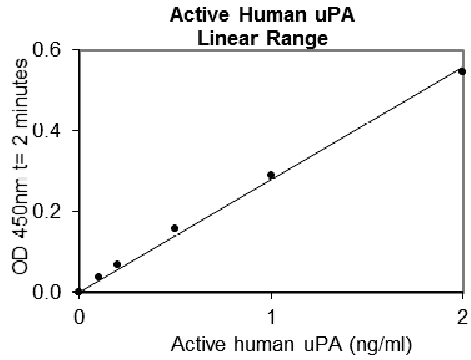
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of active uPA in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of active uPA in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

The mean value of uPA in plasma of healthy donors was found 1.1 ± 0.3 ng/ml [6].

Abnormalities in uPA levels have been reported in the following conditions:

- Breast Cancer: Increased levels of uPA have been reported in patients with breast cancer compared to healthy donors [6].
- Liver Cancer: The detection of liver cancer increased when combining the information of two tumor markers, uPA and alpha-fetoprotein [7].
- Pancreatic Cancer: Increased uPA levels may serve as a prognostic marker in human pancreatic cancer [8,9].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty-two zero standard replicates (range OD_{450}: 0.056-0.064) and calculating the corresponding concentration. The MDD was 0.013 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.50	4.05	7.92
Standard Deviation	0.023	0.065	0.50
CV (%)	4.61	1.60	6.27

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.42	0.70	3.88
Standard Deviation	0.024	0.039	0.286
CV (%)	5.70	5.62	7.37

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.56	1.06	3.16	6.31
Average % Recovery	112	106	90	90
Range	109-115%	103-108%	88-91%	87-92%

Linearity: To assess the linearity of the assay, single donor human urine samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	114	116	118	117
Range	112-116%	112-119%	114-120%	112-123%

Specificity: This assay recognizes natural and recombinant active human uPA. Pooled normal plasma from mouse, rat, dog, pig and sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit and horse resulted in significant color development.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (ng/mL)
Human Urine	1:4	54
	1:8	59
	1:16	59

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

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Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml		
B	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml		
C												
D												
E												
F												
G												
H												