REFERENCES

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Human uPA Immunoassay

Catalog Number:SEKH-0337

For the quantitative determination of human uPA concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of uPA in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	97	102
	Range(%)	86-108	94-111
1:4	Average% of Expected	95	93
	Range(%)	89-106	83-105

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 31 pg/mL

SPECIFICITY: This assay recognizes both natural and recombinant human uPA. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
Plasminogen		
Thrombin		
tPA		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of I uPA spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of uPA in two matrices

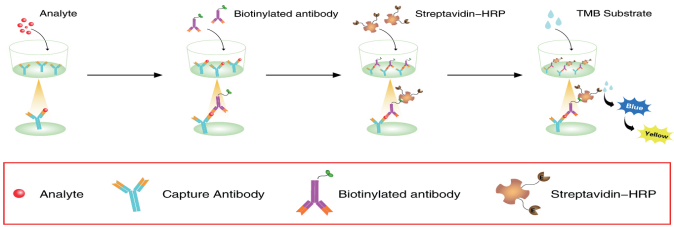
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	95	87-103
Cell culture supernatants	101	92-112

BACKGROUND

u-Plasminogen Activator (uPA) is a serine protease that converts plasminogen to plasmin, with roles in a variety of normal and pathological processes that include cell migration and tissue destruction. uPA is a potent marker of invasion and metastasis in a variety of human cancers including breast, stomach, colon, bladder, ovarian, brain, and endometrium.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for uPA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any uPA present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for uPA is added to detect the captured uPA protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

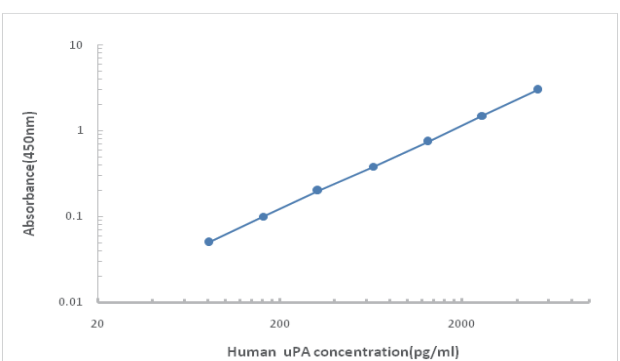
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the uPA ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.047	0.047	-
62.50	0.160	0.150	0.155	0.108
125.0	0.233	0.218	0.226	0.178
250	0.347	0.325	0.336	0.288
500	0.551	0.516	0.533	0.486
1000	0.894	0.838	0.866	0.819
2000	1.456	1.364	1.410	1.363
4000	2.372	2.223	2.297	2.250



Representative standard curve for uPA ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed,wash the plate 3 times before the assay.

⇩

Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human uPA antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-duck IgG antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 10-30 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the uPA concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack.Reseal along entire edge of the zip-seal.May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,4000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

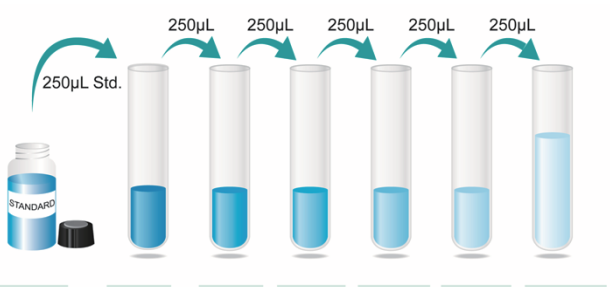
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma -Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard**Sample - - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 4000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250uL of Standard/Sample Diluent into 2000pg/ml tube and the remaining tubes. Use the stock solution of 4000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 4000 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Biotin-Conjugate anti-human uPA antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of IL–20 standard dilutions