

Human UPAR Immunoassay

Catalog Number:SEKH-0336

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

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

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TABLE OF CONTENTS

SECTION	PAGE
BACKGROUND.....	01
PRINCIPLE OF THE ASSAY.....	01
TECHNICAL HINTS AND LIMITATIONS.....	02
PRECAUTIONS.....	02
KIT COMPONENTS& STORAGE CONDITIONS.....	03
OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED.....	04
SPECIMEN COLLECTION & STORAGE.....	04
REAGENTS PREPARATION.....	04
ASSAY PROCEDURE.....	06
CALCULATION OF RESULTS.....	06
PERFORMANCE CHARACTERISTICS.....	08
REFERENCES.....	10

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of UPAR 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	95	90
	Range(%)	81-107	83-105
1:4	Average% of Expected	93	93
	Range(%)	85-106	88-114

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 10pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human UPAR. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

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

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

Recovery of UPAR in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	92	83-105
Cell culture supernatants	97	88-111

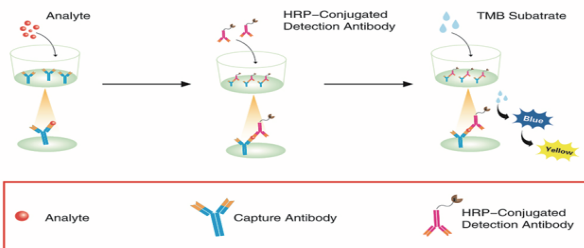
BACKGROUND

Urokinase plasminogen activator (uPA) and/or its receptor (uPAR) are essential for metastasis, and overexpression of these molecules is strongly correlated with poor prognosis in a variety of malignant tumours. uPAR and uPA levels in both resected tumor tissue and plasma are of independent prognostic significance for patient survival in several types of human cancer. This system has classically been thought to drive tumor progression by mediating directed extracellular proteolysis on the surface of migrating or invading cells, and intervening with this proteolysis by targeting uPAR has been proposed to represent a novel approach for inhibiting tumor progression. uPAR, also known as PLAUR or CD87, has been implicated in the growth, metastasis, and angiogenesis of several solid and hematologic malignancies. uPAR is a highly glycosylated, 55-60kDa integral membrane protein linked to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for UPAR has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any UPAR present is captured by the coated antibody after incubation. Following extensive washing, a HRP-conjugated antibody specific for UPAR is added to detect the captured UPAR protein in sample. The wells are then washed to remove unbound HRP-labeled antibody and Tetramethyl-benzidine (TMB) reagent is added. Incubated at room temperature, only those wells that contain UPAR, HRP-labeled antibody will appear blue in color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450nm. The OD value is proportional to the concentration of Human UPAR. You can calculate the concentration of Human UPAR in the sample by comparing the OD of the sample to the standard curve

DESCRIPTION



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.

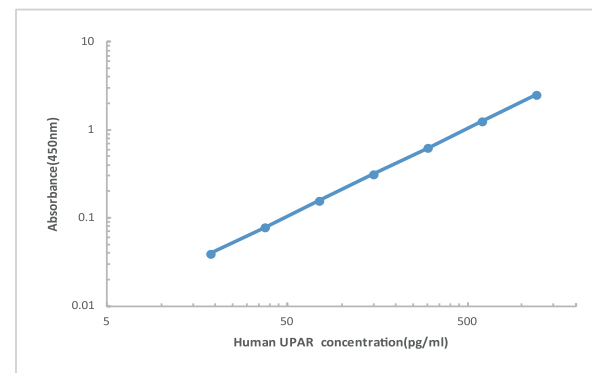
DESCRIPTION

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 UPAR ELISA

Standardized (pg/ml)	OD.	OD.	Average	Corrected
0	0.021	0.021	0.021	-
18.75	0.174	0.173	0.174	0.152
37.5	0.253	0.252	0.253	0.231
75	0.376	0.375	0.376	0.354
150	0.598	0.595	0.597	0.575
300	0.971	0.967	0.969	0.948
600	1.581	1.575	1.578	1.557
1200	2.576	2.566	2.571	2.550



Representative standard curve for UPAR 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°C).



Aspirate and wash 4 times

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



Aspirate and wash 4 times

Add 100µl Substrate solution to each well, incubate 10-20 minutes (depending on signal) at room temperature(25±2°C). Protect from light.



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

Note: oscillatory reaction at room temperature 400r

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 UPAR 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°C**
Standard - lyophilized, 1200 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
HRP Conjugated Antibody (100 X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
HRP Conjugated Diluent - 16ml/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. Squirrt 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 $\leq -20^{\circ}\text{C}$. 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°C. Centrifuge approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. 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 $\leq -20^{\circ}\text{C}$. 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

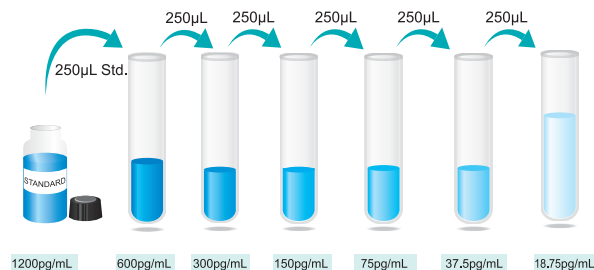
1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer,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

3. **Standard\Sample** - Reconstitute the Standard with 0.5 mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 1200pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 μL of Standard/Sample Diluent into 600pg/ml tube and the remaining tubes. Use the stock solution of 1200pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1200pg/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°C . Diluted standard shall not be reused.**

4. **Working solution of HRP-Congugated Antibody(100*):** Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100 μL of HRP Conjugate to 10mL of HRP-Congugated Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. **DO NOT FREEZE**

***The working solution should be used within one day after dilution.**



Preparation of UPAR standard dilutions