Version 25-02/25

User's Manual

Human suPAR ELISA Kit

REF DEIA-BV1

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human suPAR ELISAKit is a sandwich enzyme immunoassay for the quantitative measurement of human soluble urokinase-type plasminogen activator receptor (suPAR) in serum, plasma (EDTA, citrate, heparin) and urine.

General Description

The urokinase-type plasminogen activator system consists of a protease, a receptor (uPAR) and inhibitors. uPAR was initially characterized as a cofactor for plasminogen activation by its ligand urokinase-type plasminogen activator (uPA or urokinase).

Structurally, uPAR is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein encoded by PLAUR gene. It consists of three homologous domains (DI, DII and DIII), each of approximately 90amino acids. The molecular mass of non-glycosylated uPAR is approximately 35 kDa, whereas glycosylated uPAR has a molecular mass of approximately 60 kDa. Removal of the GPI anchor by phospholipases or extracellular proteolytic cleavage yields a soluble form - soluble urokinase-type plasminogen activator receptor (suPAR). Cleavage of uPAR from the cell can occur both at the GPI-anchor and at the linker region between DI and DII. Thus, suPAR is a circulating protein ranging from 20 to 50 kDa, depending on the degree of glycosylation and proteolytic cleavage.

The uPAR has been shown to associate with many signalling molecules and to mediate signal transduction. Chemotaxis-inducing molecules upregulate uPAR in different cell types, including neutrophils, macrophages, lymphocytes, endothelial cells and malignant cells. uPAR promotes the migration and adhesion of leucocytes by binding to β-integrins. Moreover, uPAR has a pivotal role in cell proliferation, angiogenesis and fibrinolysis.

After cleavage from the cell surface, soluble uPAR can be measured in the blood and other organic fluids such as urine, saliva, bronchoalveolar lavage (BALF) and cerebrospinal fluid (CSF). In such matrices suPAR, similarly to anchored uPAR, also takes part in various immunological functions, including cell adhesion, migration, chemotaxis, proteolysis, immune activation, tissue remodelling, cell invasion and signal transduction.

Principles of Testing

In the Human suPAR ELISA Kit, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-human uPAR(suPAR) antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-human uPAR(suPAR) antibody is added and incubated with the captured suPAR for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of suPAR. A standard curve is constructed by plotting absorbance values against suPAR concentrations of standards and concentrations of unknown samples are determined using this standard curve.

Reagents And Materials Provided

- Antibody Coated Microtiter Strips: ready to use, 96 wells
- Biotin Labelled Antibody: lyophilized, 2 vials
- Streptavidin-HRP Conjugate: ready to use, 13 ml
- Master Standard: lyophilized, 2 vials
- Dilution Buffer: ready to use, 50 ml
- Wash Solution Conc. (10x): concentrated, 100 ml
- Substrate Solution: ready to use, 13 ml
- Stop Solution: ready to use, 13 ml
- Product Data Sheet + Certificate of Analysis

Materials Required But Not Supplied

_ Deionized (distilled) water

_ T est tubes for diluting samples

_ Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)

_ Precision pipettes to deliver 5-1000 µl with disposable tips

_ Multichannel pipette to deliver 100 µl with disposable tips

_ Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing

_ Vortex mixer

_ Orbital microplate shaker capable of approximately 300 rpm

_ Microplate washer (optional). [Manual washing is possible but not preferable.]

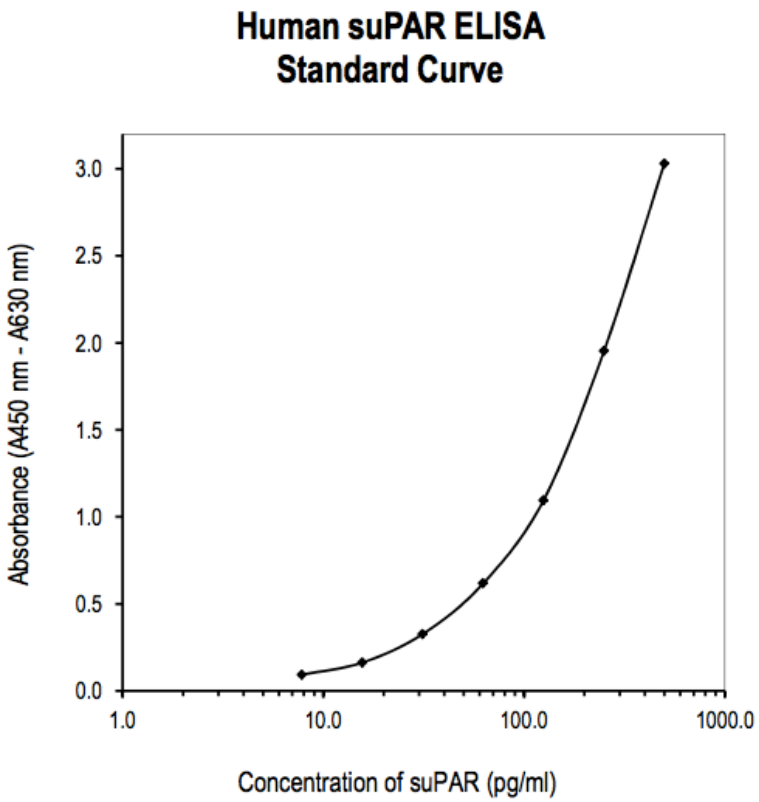
_ Microplate reader with 450±10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)

_ Software package facilitating data generation and analysis (optional)

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Typical Standard Curve



Precision

Intra-assay (Within-Run) (n=8)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
Serum 1	2101.8	108.4	5.2
Serum 2	969.1	51.3	5.3

Inter-assay (Run-to-Run) (n=6)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
Serum 1	2143.1	116.6	5.4
Serum 2	1185.9	75.5	6.4