AssayMax Human Urokinase Receptor (uPAR) ELISA Kit

Catalog Number EU1002-1
Lot #

Introduction

Urokinase-type plasminogen activator receptor (uPAR) is linked to the cell membrane via a glycosylphosphatidylinositol anchor attached to the *C*-terminal hydrophobic domain (1). Both single chain pro-uPA and two chain uPA bind to the NH_2-terimnal domain of uPAR (2). When bound by uPA, uPAR plays a major role in local proteolytic processes, thus facilitating cell migration as may occur during angiogenesis (3), neointima and atherosclerotic plaque formation (4), and tumor cell invasion (5). uPAR may be a multifunctional receptor, not only promoting pericellular proteolysis but also involved in integrin-mediated cell adhesion and migration (6). uPAR has also been implicated in intracellular signaling, cellular differentiation, growth and chemotaxis (7). The expression of both u-PA and uPAR is increased in human atherosclerotic plaque (8) and arterial aneurysms (9).

Principal of the Assay

The AssayMax Human uPAR ELISA kit is designed for detection of uPAR in human plasma, tissue extracts, cell culture supernatants and urine. This assay employs a quantitative sandwich enzyme immunoassay technique that measures uPAR in 3.5 hours. A murine monoclonal antibody specific for uPAR has been pre-coated onto a microplate. uPAR in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for uPAR, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **uPAR Microplate:** One 96-well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against human recombinant uPAR.

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **uPAR Standard:** Recombinant human uPAR in a buffered protein base (20 ng, lyophilized).
- **Biotinylated uPAR Antibody (100x):** A 100-fold biotinylated polyclonal antibody against uPAR (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (120 µl).
- **EIA Diluent Concentrate (10x)**: A buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A stabilized chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel pipettes)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000 x g for 10 minutes and assay. Dilute samples 1:5 with EIA Diluent by adding 30 µl of sample to 120 µl of EIA Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Diluent supernatants 1:5 with EIA Diluent and assay. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14,000 x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:5 into EIA Diluent and assay. The undiluted samples can be stored at -20^{0}C or below.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:5 into EIA Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the 20 ng of human uPAR Standard with 2.5 ml of EIA Diluent to produce an 8 ng/ml of stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially

diluting the standard solution (8 ng/ml) twofold with equal volume of EIA Diluent to produce 4, 2, 1, 0.5, 0.25, and 0.125 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[uPAR] (ng/ml)
P1	1 part Standard (8 ng/ml) + 1 part EIA Diluent	4.000
P2	1 part P1 + 1 part EIA Diluent	2.000
P3	1 part P2 + 1 part EIA Diluent	1.000
P4	1 part P3 + 1 part EIA Diluent	0.500
P5	1 part P4 + 1 part EIA Diluent	0.250
P6	1 part P5 + 1 part EIA Diluent	0.125
P7	1 part EIA Diluent	0.000

- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water.
- **Biotinylated uPAR Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (25 - 30^0C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely and store in a vacuum desiccator to minimize exposure to water vapor.
- Add 50 µl of standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit the plate 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated uPAR Antibody Conjugate per well and incubate for 30 minutes.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution per well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

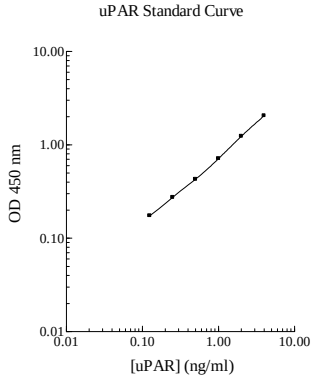
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.

- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis of the 4-parameter or log-log curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the sample value by the dilution factor of 5.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Sensitivity and Specificity

- The minimum detectable dose of uPAR is typically < 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were were 5.3 % and 6.2% respectively.
- This assay recognizes both natural and recombinant human uPAR as well as uPA/uPAR complex. No significant cross-reactivity or interference was observed.

References

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