Human uPA
ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

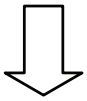
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

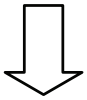
Thank you for choosing Assaypro.

Assay Summary

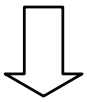
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



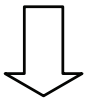
Wash, then add
50 µl of Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add
50 µl of SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 15 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Human Urokinase-type Plasminogen Activator (uPA) ELISA Kit

Catalog No. EU1001-1
Sample Insert/Reference Only

Introduction

Urokinase-type plasminogen activator (uPA) is a highly restricted serine protease that converts the zymogen plasminogen to active plasmin, a broad-spectrum serine proteinase capable of degrading most of the major protein components of the extracellular matrix. Binding of uPA to its receptor and subsequent uPA mediated pericellular proteolysis are involved in many processes including cell migration and tissue remodeling in angiogenesis, atherogenesis, tumor cell metastasis, and ovulation (1, 2). A high level of uPA is a marker associated with a poor prognosis for aggressive breast cancer, aggressive prostate cancer, bladder cancer, and gastric cancer (3-5).

Principle of the Assay

The AssayMax Human Urokinase-type Plasminogen Activator (uPA) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human uPA in plasma, serum, tissue, milk, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures uPA in less than 4 hours. A polyclonal antibody specific for uPA has been pre-coated onto a 96-well microplate with removable strips. Urokinase in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for uPA, 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

- **Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**

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

Reagents

- **Human uPA Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against uPA.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human uPA Standard:** Human uPA in a buffered protein base (24 ng, lyophilized).
- **Biotinylated Human uPA Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human uPA (80 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- 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 3000 x *g* for 10 minutes and use supernatants. Dilute samples 1:2 with MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:2 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:4 into MIX Diluent or within the range of 2x - 20x, and assay. The undiluted samples can be stored at -20°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 14000 x *g* for 30 minutes. Collect the supernatant, measure the protein concentration, dilute if necessary, and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Samples can be stored at -20°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.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 24 ng of Human uPA Standard with 3 ml of MIX Diluent to generate an 8 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (8 ng/ml) 1:2 with MIX Diluent to produce 4, 2, 1, 0.5, and 0.25 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[uPA] (ng/ml)
P1	Standard (8 ng/ml)	8.00
P2	1 part P1 + 1 part MIX Diluent	4.00
P3	1 part P2 + 1 part MIX Diluent	2.00
P4	1 part P3 + 1 part MIX Diluent	1.00
P5	1 part P4 + 1 part MIX Diluent	0.50
P6	1 part P5 + 1 part MIX Diluent	0.25
P7	MIX Diluent	0.00

- **Biotinylated Human uPA Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human uPA Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human uPA Antibody to each well and incubate for 1 hour.
- Wash the microplate as described 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 the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 15 minutes or till the optimal blue color density develops. Gently tap the plate to

ensure thorough mixing and break the bubbles in the well with pipette tip.

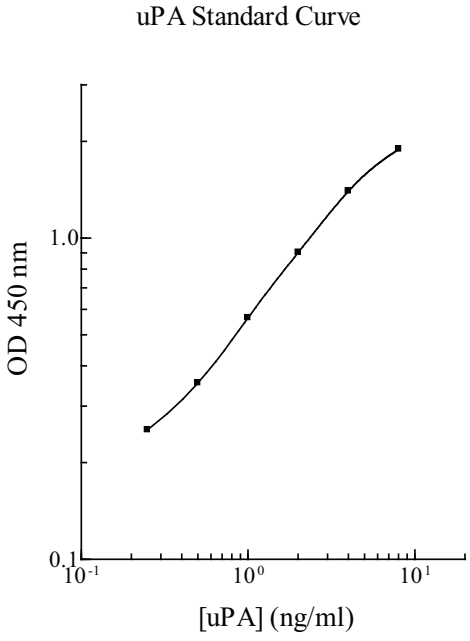
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. 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 duplicate or 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 using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

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



Performance Characteristics

- The minimum detectable level of uPA is typically ~ 0.2 ng/ml
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.2% respectively.
- This assay recognizes single chain, two-chain, and both receptor and PAI-bound human uPA.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
No Dilution	89%	92%
1:2	98%	96%
1:4	102%	104%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:2	86%
1:4	97%
1:8	104%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
No dilution	94%
1:2	92%
1:4	99%

Recovery

Standard Added Value	0.3 – 3 ng/ml
Recovery %	88 – 112%
Average Recovery %	98.5%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	50%
Mouse	20%
Rat	20%
Swine	80%
Rabbit	None

References

(1) Okada, S. *et al.* (1996) *Arterioscl. Thromb. Vasc. Biol*. 16: 1269
(2) Besser, D. *et al.* (1996) *Fibrinolysis* 10: 215
(3) Duffy, M.J. *et al.* (1990) *Cancer Res*. 50:6827
(4) Hasui, Y. *et al.* (1992) *Int. J. Cancer* 50: 871
(5) Nishino, N. *et al.* (1988) *Thromb. Res*. 50:527

Version 8.1