Human uPA/Urokinase ELISA Kit

Cat. No.: H1509988 | Pack size: 96T | Storage: 2-8°C

Product Information

Item	Details
Available Pack Size(s)	96T
Specifications & Purity	BioReagent, for enzyme immunoassay (ELISA)
Stability and Storage	Each component has a shelf life of 6 months under corresponding storage conditions.
Storage Conditions	Store at 2-8°C
Shipped In	Wet ice
Application	ELISA
Sample type	body fluids, cell culture supernatants, Plasma, Serum, tissue homogenates
Detection Range	15.62 pg/mL~1,000 pg/mL
Method of Detection	Sandwich ELISA
Detected Species Object	Human
Specificity	Detecting Human uPA/Urokinase in samples, with no significant cross-reactivity to other homologs.
Detection Instrument	Microplate reader
Detection Wavelength	450nm

Product Description

This kit adopts the double-antibody sandwich ELISA method to detect human uPA concentration in samples. Human uPA capture antibodies are pre-coated on microplates. When samples or standards are added, human uPA binds specifically to capture antibodies, while unbound components are removed by washing. Subsequently, biotin-labeled human uPA antibodies are added to form sandwich immune complexes with uPA, and excess free reagents are washed away. Enzyme

conjugates are then added, where biotin binds specifically to the enzyme conjugate, linking HRP on the conjugate to the immune complex. Unbound substances are washed again. Finally, TMB substrate is added. If human uPA is present in the sample, HRP catalyzes colorless substrate to produce blue color. After stop solution is added, the reaction turns yellow. OD values at 450 nm are measured by a microplate reader. Human uPA concentration is positively correlated with OD450. The sample concentration can be calculated by comparing sample OD values with the standard curve established from standards.

Contents & Storage

Item No.	Appearance	Components	96T	Storage
H1509988A	-	Pre-coated Assay Plate	96 wells	2-8℃.
H1509988B	Liquid	Sample Diluent	30 mL	2-8℃.
H1509988C	Solid	Recombinant human uPA standard (lyophilized)	2 vials (10 ng/vial)	2-8℃.
H1509988D	Liquid	Biotin-labeled human uPA antibody	130 µL (Dilution ratio: 1:100)	2-8℃.
H1509988E	Liquid	Antibody Diluent	12 mL	2-8℃.
H1509988F	Liquid	Enzyme Conjugate (HRP-labeled Streptavidin)	130 µL (Dilution ratio: 1:100)	2-8℃.
H1509988G	Liquid	Enzyme Conjugate Diluent	12 mL	2-8℃.
H1509988H	Liquid	Concentrated Washing Buffer (25×)	30 mL	2-8℃.
H1509988I	Liquid	TMB Substrate	10 mL	2-8℃.
H1509988J	Liquid	Stop Solution	10 mL	2-8℃.
H1509988K	-	Plate Sealer	4 sheets	2-8℃.

Assay Procedures

Sample Preparation

1. Select appropriate pretreatment according to sample type:

A. Cell supernatant: Centrifuge cell culture supernatant at 100-500×g for 5 min to remove suspended particles.

B. Serum sample: Allow whole blood to stand at room temperature for 0.5-2 h for natural coagulation. Centrifuge at 1000-2000×g for 10 min at 4 ℃ and collect yellow supernatant. Do not aspirate precipitates. Keep serum on ice. Do not add preservatives or anticoagulants.

C. Plasma sample: Anticoagulate whole blood with EDTA, mix well and keep on ice. Centrifuge at 1000-2000×g for 10 min at 4 ℃ and collect supernatant. Avoid precipitates and keep plasma on ice.

D. Tissue homogenate & body fluid: Centrifuge to remove sediment.

Notes:

① If samples cannot be tested immediately, aliquot and store at -20 ℃. Avoid repeated freeze-thaw cycles.

② Samples must be clear. Centrifuge to remove turbidity before detection.

③ Hemolyzed, lipemic, icteric or contaminated samples are not recommended.

2. Sample Dilution

Estimate target concentration according to literature and choose proper dilution ratio to keep samples within optimal detection range.

① 10-100 ng/mL: Dilute 1:100 (add 3 µL sample into 297 µL sample diluent).

② 1-10 ng/mL: Dilute 1:10 (add 25 µL sample into 225 µL sample diluent).

③ 15.62-1000 pg/mL: Dilute 1:2 (add 100 µL sample into 100 µL sample diluent).

④ ≤15.62 pg/mL: No dilution required.

Above protocols are for reference only. Record detailed dilution procedures.

Pre-assay Preparation

1. Equilibrate kit to room temperature for 20 min after taking out from 4 ℃. For reagents from -20 ℃, fully thaw then equilibrate for 20 min. Store remaining reagents at 4 ℃ or -20 ℃ after assay.
2. Dilute 25× concentrated washing buffer to 1× working buffer with distilled/deionized water.
3. Dilution and Use of Recombinant Human uPA Standard(Prepare within 2 hours before use; operate at room temperature and strictly control the temperature at 25-28℃).

① Prepare 10 ng/mL standard: Add 1 mL sample diluent into standard vial, stand for ≥15 min, mix thoroughly.

② Prepare 1000 pg/mL standard: Add 100 µL 10 ng/mL standard into 900 µL diluent, mix and label.

③ Serial two-fold dilution of 1000 pg/mL standard as below (0 pg/mL=diluent blank).

Tube No.	Diluent Volume (µL)	Reconstituted Standard Volume (µL)	Final Standard Concentration (pg/mL)

Tube No.	Diluent Volume (μL)	Reconstituted Standard Volume (μL)	Final Standard Concentration (pg/mL)
A	0	1000	1000
B	300	300 (from Tube A)	500
C	300	300 (from Tube B)	250
D	300	300 (from Tube C)	125
E	300	300 (from Tube D)	62.5
F	300	300 (from Tube E)	31.25
G	300	300 (from Tube F)	15.62
H	300	0	0

Note: Discard any remaining reconstituted standard after pipetting.

4. Prepare biotin-antibody working solution:

①Calculate total volume (add extra 100-200 μL for loss). Use 100 μL per well.

②Dilute antibody at 1:99 (1 μL antibody + 99 μL diluent), mix gently.

5. Prepare enzyme conjugate working solution (prepare within 1 h before use):

①Calculate total volume (add extra 100-200 μL). Use 100 μL per well.

②Dilute conjugate at 1:99 (1 μL enzyme conjugate + 99 μL diluent), mix gently.

Detection Steps

1. Take required strips, assemble into frame. Seal unused strips in aluminum pouch and store at 4 ℃ / -20 ℃.

Note: ①Duplicate wells are recommended for both standards and samples.

②A standard curve must be established for each independent assay.

2. Add 100 μL diluted samples and standards into corresponding wells. Seal plate and incubate at 37 ℃ for 90 min.

Note:

① Please refer to relevant literature to estimate the approximate concentration of the target protein in samples. If the concentration exceeds the maximum value of the kit standard curve, dilute the sample appropriately before detection.

② The entire sample adding procedure shall be completed within 10 minutes, otherwise the test results may be affected.

3. Discard liquid, blot dry on absorbent paper without washing.

4. Add 100 μL biotin-antibody working solution per well, seal and incubate at 37 ℃ for 60 min.

5. Wash plate 5 times with 300 µL 1× buffer per well, interval 15-30 s, then blot dry. Insufficient washing causes large errors.
6. Add 100 µL enzyme conjugate per well, seal and incubate at 37 ℃ for 30 min in dark.
7. Wash plate 5 times as step 5.
8. Add TMB substrate solution (100 µL per well), seal the wells with plate sealing film, and incubate at 37 ℃ for 10-25 minutes away from light.

Note:

① Avoid contact between TMB and oxidants or metals during storage and operation.

② The optimal color development time varies with laboratory conditions. Distinct gradient blue color can be visually observed in the first 3–4 wells of standards when the reaction is complete.

9. Add stop solution (100 µL per well). Mix well immediately and measure OD_{450} with a microplate reader. Set 540 nm or 570 nm as reference wavelength to calculate corrected absorbance (OD_{450}-OD_{540} or OD_{450}-OD_{570}).

Note: OD reading should be completed within 10 minutes.

Data Analysis

(1) Establish the standard curve. Take standard concentration as the X-axis and OD value as the Y-axis. Fit the curve with four-parameter logistic (4-PL) regression using software. The sample concentration can be calculated according to its OD value on the standard curve.

Note:

① Results are valid only when the OD difference between duplicate wells is within 20%. The average OD value of duplicates is adopted for calculation.

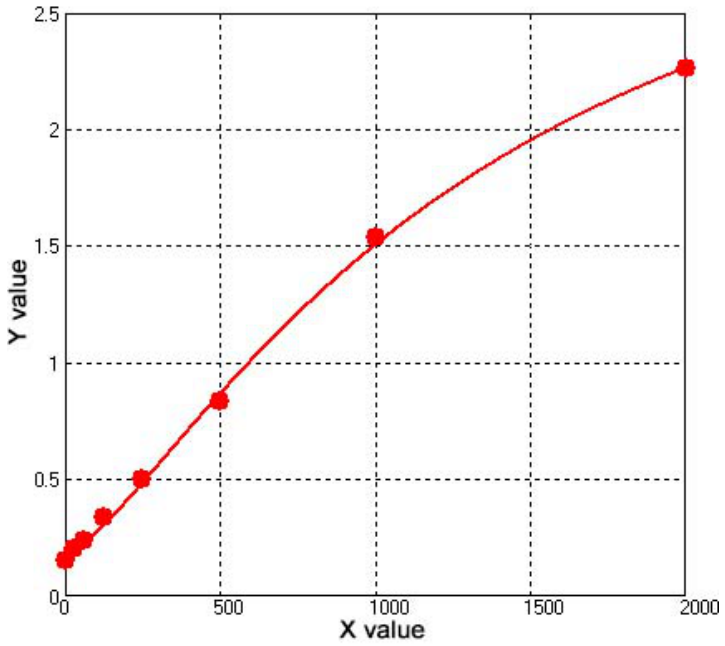
②If sample OD exceeds the upper limit of the standard curve, dilute the sample and retest. Multiply the final concentration by the dilution factor.

(2) Typical Data:

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard concentration (pg/ml)	O.D.
0	0.152
15.63	0.201
31.25	0.236
62.5	0.335
125	0.498

Standard concentration (pg/ml)	O.D.
250	0.837
500	1.535
1000	2.262



(3) Precision:

Intra-Assay Precision (precision within an assay) was assessed by testing three samples of known concentration twenty times on one plate. Inter-Assay Precision (precision between assays) was assessed by testing three samples in separate assays performed by at least three technicians using two lots of components.

Sample	Intra-Assay 1	Intra-Assay 2	Intra-Assay 3	Inter-Assay 1	Inter-Assay 2	Inter-Assay 3
n	20	20	20	20	20	20
Mean (pg/ml)	61	163	59	59	62	91
Standard Deviation	1.5	5.5	2.4	2.4	3.2	5.1
CV%	2.4	3.3	4	4	5.1	5.6

(4) Recovery:

The recovery of human uPA/Urokinase spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average Recovery (%)	Range (%)
Cell culture supernates (n=10)	101	94%-119%
Serum (n=9)	94	89%-112%
Herparin plasma (n=9)	99	90%-114%

(5) Linearity:

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human uPA/Urokinase were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

Dilution Ratio	Index	Cell Culture Supernatants (n=10)	Serum (n=10)	Heparin Plasma (n=10)
1:4	Average of Expected (%)	105	90	101
1:4	Range (%)	83-120	83-97	93-119
1:8	Average of Expected (%)	91	100	95
1:8	Range (%)	86-106	96-102	89-118
1:16	Average of Expected (%)	102	90	104
1:16	Range (%)	82-108	88-94	92-111
1:32	Average of Expected (%)	100	91	102
1:32	Range (%)	90-104	86-99	96-115

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