Rat Plasminogen Activator, Urokinase (uPA)

Enzyme-linked Immunosorbent Assay Kit

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

Instruction Manual

- **Catalog No:** JL11073
- **Detection Range:** 15.62-1000pg/mL
- **Storage:** 2-8℃/-20℃
- **Size:** 96T/48T
- **Shelf life:** 6 months
- **Species:** Rat

Please refer to the specific shelf life on the outer package label of the kit.

uPA:

Urokinase, also known as urokinase-type plasminogen activator (uPA), is a serine protease present in humans and other animals. The human urokinase protein was discovered, but not named, by McFarlane and Pilling in 1947.[5] Urokinase was originally isolated from human urine, and it is also present in the blood and in the extracellular matrix of many tissues. The primary physiological substrate of this enzyme is plasminogen, which is an inactive form (zymogen) of the serine protea se plasmin. Activation of plasmin triggers a proteolytic cascade that, depending o n the physiological environment, participates in thrombolysis or extracellular mat rix degradation. This cascade had been involved in vascular diseases and cancer progression.

Test Principle:

This assay employs the sandwich enzyme immunoassay technique. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat uPA. Add Sample, Standard, Biotin-antibody, and Streptavidin-HRP by turn, with incubation and washing. TMB Substrate is added for color development, and TMB will appear blue in color catalyzed by HRP and to a final yellow color in reaction to acid. The intensity of the color is positive to the Rat uPA in the sample. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. You can calculate the concentration of Rat uPA in the samples by comparing the OD of the samples to the standard curve.

Sensitivity：5.72pg/mL

Specificity：This kit recognizes Rat uPA in samples. No significant cross-reactivity or interference between Rat uPA and analogues was observed.

Kit Components & Storage:

Reagents	96T	48T	Storage
Pre-coated Assay Plate	8×12 strips	8×6 strips	-20℃, 6 months
Standard	2 vials	1 vial	-20℃, 6 months
Universal Diluent	2×20mL	1×20mL	2-8℃, 6 months
Biotin-antibody (100×)	120μL	60μL	-20℃, 6 months
Streptavidin-HRP (100×)	120μL	60μL	2-8℃(Away from light), 6 months
Wash Buffer (20×)	2×10mL	1×10mL	2-8℃, 6 months
TMB Substrate	10mL	5mL	2-8℃(Away from light), 6 months
Stop Solution	6mL	3mL	2-8℃, 6 months
Plate Sealer	4 pieces	4 pieces	-
Instruction Manual	1 copy	1 copy	-

Storage Tips:

An unopened kit can be stored at 2-8°C for 1 month.

If the kit is not supposed to be used up within 1 month, please store the Pre-coated Assay Plate, Standard, and Biotin-antibody(100×) at -20°C, other reagents at 2-8°C.

Shelf life is 6 months. Avoid repeated freeze-thaw cycles.

Other Supplies Required:

1. Microplate reader with 450nm wavelength filter.
2. High-precision transfer pipette, EP tubes and disposable pipette tips.
3. Incubator capable of maintaining 37℃.
4. Deionized or distilled water.

Note:

1. Please wear lab coats, eye protection, and latex gloves for protection.
2. Strictly follow the specified time and temperature for incubation to ensure accurate results. Bring all reagents to room temperature (20-25℃) before use. Refrigerate reagents immediately after use.
3. Incorrect plate washing may lead to inaccurate results. Ensure that the wells are aspirated before adding TMB Substrate. Avoid prolonged drying of the wells during the whole process.
4. Clean liquid and fingerprints from the bottom of the microplate to avoid affecting the OD value.
5. TMB Substrate should be colorless and not be used if the color turns blue.
6. Avoid cross-contamination of reagents and samples to avoid false results.
7. Avoid direct exposure to strong light during storage and incubation.
8. Any reagents cannot be exposed to bleaching solvents or strong gases released from bleaching solvents to avoid destroying the biological activity of the reagents.
9. Do not use expired products, and do not mix components of different catalog numbers and lot numbers.
10. Recombinant proteins from sources outside the kit may not be recognized due to the mismatching with the antibody.
11. If disease transmission is possible, all samples must be managed properly. Samples and devices must be handled according to established procedures.

Samples Collection and Storage:

1. **The detection range of the kit is not equal to the concentration range of the target substance in the sample.** Predict the concentration of the target substance in the sample from relevant literature and determine the actual concentration of the sample by pre-test. If the concentration of the target substance in the sample is too high or too low, please dilute or concentrate the sample appropriately.
2. If the sample type is not included in the manual, a pre-test is suggested to verify the validity.
3. **Serum:** Whole blood samples be collected in serum separator tubes, clotted for 2 hours at room temperature or overnight at 2-8°C, and then centrifuged at 1000 × g for 20 minutes. Remove serum and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze-thaw cycles.
4. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 × g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze-thaw cycles.
5. **Tissue homogenates:** Rinse the tissue with pre-cooled PBS (0.01M, pH =7.4) to remove excess blood (lysed erythrocytes in the homogenate will affect the results), weigh the tissue, and mince into small pieces. Add the minced tissue to the corresponding volume of PBS into a glass homogenizer and grind on ice or in a homogenizer. (tissue weight (g): PBS (mL) volume=1:9) The specific volume can be adjusted according to the needs of the experiment, and make a record. Add proteinase inhibitors in PBS are recommended. For further lysis of tissue cells, the homogenate can be broken by ultrasonication or repeatedly frozen and thawed. Finally, the homogenate is centrifuged at 5000 × g for 5-10 min to get the supernatant.

6. **Cell culture supernates:** Centrifuge samples for 20 minutes at 1,000×g. Collect the supernatant and assay it immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze-thaw cycles.
7. **Cell lysates:** Washed adherent cells with pre-cooled PBS, then detached with trypsin and centrifuged at 1000 × g for 5 minutes to collect the cells; suspended cells can be collected by centrifugation directly. Collected cells were washed three times with pre-cooled PBS, resuspended in 150-200 μL of PBS per 1 × 10^6 cells (Add proteinase inhibitors in PBS are recommended; Reduce the volume of PBS appropriately if the content is very low), and broken by repeatedly frozen and thawed or sonication. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cell fragments. Collect the supernatant, assay immediately, or store in aliquots at ≤ -20°C.
8. **Other biological fluids:** Centrifuge samples for 20 minutes at 1,000×g. Collect the supernatant and assay it immediately.
9. **Sample appearance:** Samples should be clear and transparent, and suspended material should be removed by centrifugation.
10. **Precautions for samples:** Samples should be assayed within 7 days when stored at 4℃, otherwise they must be separated and stored at -20℃ (≤1 month) or -80℃ (≤6 months). Avoid repeated freeze-thaw cycles. Sample hemolysis will influence the result, so it should not be used.

Sample Dilution Method:

Please estimate the concentration range of the samples in advance. If samples need to be diluted, please refer to the dilution method below:

100 fold dilution: One-step dilution. Add 5μL sample into 495μL Universal Diluent to yield 100 fold dilution.

1000 fold dilution: Two-step dilution. Add 5μL sample into 95μL Universal Diluent to yield 20 fold dilution. Then add 5μL 20 fold diluted sample into 245μL Universal Diluent, after this the sample has been diluted at 1,000 fold.

100000 fold dilution: Three-step dilution. Add 5μL sample into 195μL Universal Diluent to yield 40 fold dilution. Then add 5μL 40 fold diluted sample into 245μL Universal Diluent to yield 50 fold dilution. Finally, add 5μL 2,000 fold diluted sample into 245μL Universal Diluent, after this the sample has been diluted at 100,000 fold dilution.

The volume of liquid taken in each step of dilution should not be less than **3μL**, and the dilution should not exceed **100 fold**. Each step of dilution should be mixed well to avoid foaming.

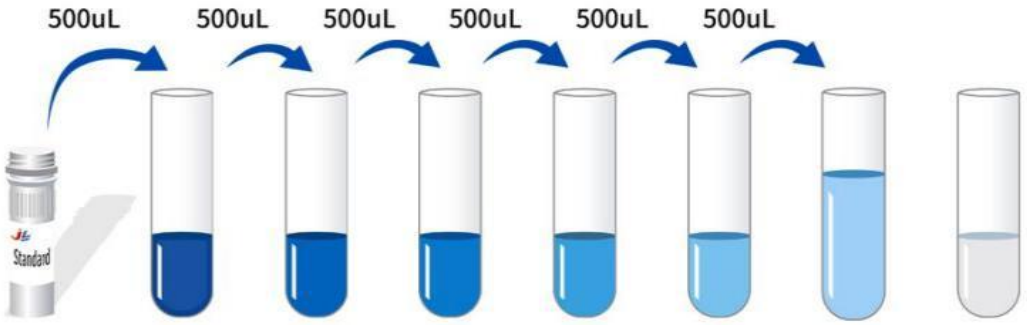
Reagent Preparation:

1. Please take the kit out of the refrigerator 10 minutes in advance and equilibrate it to room temperature.
2. **Standard working solution:** Add 1mL Universal Diluent to the lyophilized standard, keep for 15 minutes to completely dissolve, and mix gently (concentration of 1000 pg/mL). Dilute according to the following concentrations: 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.62 pg/mL, 0 pg/mL.

   Dilution method: Take 7 EP tubes, add 500μL Universal Diluent to each tube. Pipette 500μL of 1000 pg/mL standard working solution to the first EP tube and mix it well to produce 500 pg/mL standard working solution.

Pipette 500μL standard working solution from the former tube into the latter one according to this step. The illustration below is for reference.

Note: The last tube is regarded a **Blank** well, do not pipette solution into it from the former tube.



pg/mL	1000	500	250	125	62.5	31.25	15.62	0

3. **Biotin-antibody(1×) working solution:** Centrifuge the concentrated Biotin-antibody(100×) at 1000×g for 1 minute at 15 minutes before use. Dilute Biotin-antibody(100x) to 1×Working solution (Concentrated Biotin-antibody: Universal Diluent= 1: 99, e.g., 10μL Biotin-antibody+990μL Universal Diluent). The working solution should be prepared just before use.
4. **Streptavidin-HRP(1×) working solution:** Centrifuge the concentrated Streptavidin-HRP(100×) at 1000×g for 1 minute at 15 minutes before use. Dilute Streptavidin-HRP(100x) to 1×Working solution (Concentrated Streptavidin-HRP: Universal Diluent= 1: 99, e.g., 10μL Streptavidin-HRP+ 990μL Universal Diluent). The working solution should be prepared just before use.
5. **Wash Buffer(1×):** Take 10ml Wash Buffer(20×) into 190ml distilled water. The concentrated wash solution taken out of the refrigerator may have crystals, which is normal and can be placed at room temperature until the crystals are completely dissolved.

Assay Procedure:

1. Bring all kit components and samples to room temperature before use.

2. Add 100μL **Sample** or different concentrations of **Standards** into the corresponding wells, and add 100μL **Universal Diluent** to the blank well. Cover with the plate sealer. Incubate for 60 minutes at 37 ℃. **Note:** The samples to be tested should be diluted a minimum of 1x with Universal Diluent before adding to the microplate. To minimize matrix effects on the test results, and multiply by the corresponding dilution when calculating the sample concentration. It is recommended to set up duplicate wells for all samples and standards to be tested.

3. Decant the liquid from each well, do not wash. Immediately add 100μL **Biotin-antibody (1×) working solution** to each well. Cover with the plate sealer. Incubate for 60 minutes at 37°C.

4. Decant the solution from each well, add 300μL **Wash Buffer (1×)** to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. **Note:** Microplate washer can be used in this step and other wash steps.

5. Add 100μL **Streptavidin-HRP (1×) working solution** to each well. Cover with the plate sealer. Incubate for 30 min at 37°C.

6. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 4.

7. Add 90μL **TMB Substrate** to each well. Cover with the plate sealer. Incubate for 15 minutes at 37 ℃. Protect from light. The liquid will turn blue by the addition of TMB Substrate.

8. Add 50μL **Stop Solution** to each well. Conduct measurement at 450nm immediately.

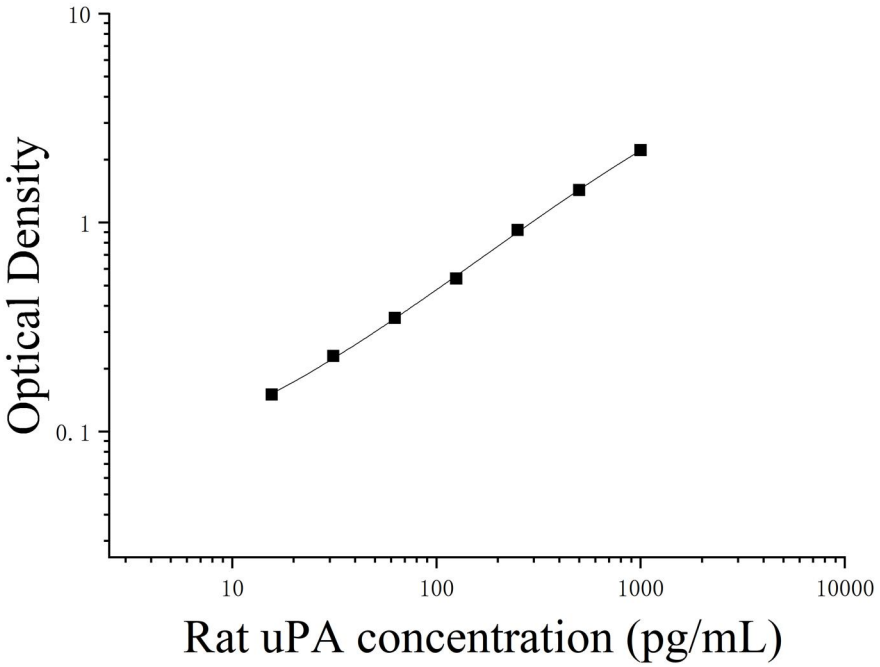
Calculation of Results:

1. Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the y-axis.
2. If the OD of the sample exceeds the upper limit of the standard curve, it should be re-test with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical Data:

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, wash technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data are provided below for reference only.

Concentration (pg/mL)	1000	500	250	125	62.5	31.25	15.62	0
OD	2.31	1.52	1.01	0.63	0.44	0.32	0.24	0.09
Corrected OD	2.22	1.43	0.92	0.54	0.35	0.23	0.15	-



Performance:

1. Repeatability: Intra-assay Precision (Precision within an assay)：CV% < 10%; Inter-assay Precision (Precision between assays)： CV% < 10%

2. Recovery: The recovery of Rat uPA spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range(%)	Average Recovery(%)
Serum(n=8)	85-101	96
Plasma(n=8)	92-105	102
Cell Culture Supernates(n=8)	96-108	104

3. Linearity: Samples were spiked with high concentrations of Rat uPA and diluted with Universal Diluent to produce samples with values within the range of the assay.

		Serum(n=4)	Plasma(n=4)	Cell Culture Supernates(n=4)
1： 2	Range (%)	85-95	88-96	90-110
	Average (%)	91	93	96
1： 4	Range (%)	89-103	87-108	105-115
	Average (%)	94	98	106

Declaration:

1. Limited by current conditions and scientific technology, there can not be comprehensive identification and analysis of all raw materials, the product may have certain quality and technical risks.
2. This kit is for research use only and we will not be responsible for any problems if used for clinical diagnosis or any related procedures.
3. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.
4. Each kit undergoes strict QC before shipment. However, due to transportation conditions, differences in laboratory equipment, and other factors, the results may be inconsistent with the factory data. Intra-assay variation between batches of kits may also occur due to the above reasons.
5. Incorrect preparation of reagents and parameter settings of the Microplate reader may cause incorrect results. Please read the instructions carefully and adjust the instrument before the experiment.

Analysis of the Problem:

If the results are not good, please take pictures in time and save data, keep the used plates and unused reagents, and then contact us to solve the problem. Meanwhile, you can also refer to the following information:

Problem	Possible causes	Solutions
Poor Standard Curve	Improper dilution of the Standard	Ensure Standard is dissolved and diluted according to the method
	Inaccurate pipetting	Check and calibrate pipettes
	Evaporation of reaction solution	Seal the plate with plate sealer
	Insufficient washing	Wash sufficiently and add sufficient Wash Buffer
	Dirty plate	Make sure that the bottom of plate is clean
Weak or no color development	Incorrect incubation time	Ensure sufficient incubation time
	Incorrect incubation temperature	Incubation at recommended temperature
	Insufficient reagent volume added	Check the pipettes and follow the procedure exactly
	Improper dilution	Check reagent dilution procedure
	HRP conjugate inactive	Mixed HRP conjugate and TMB Substrate, rapid coloring

Low OD Value	Incorrect Microplate reader Settings	Check instrument wavelength
	No Stop Solution added	Add Stop Solution
	Plate left too long before reading	Timely reading
	High sample content	Determination of the appropriate dilution by pre-test
	Low sample content	Determination of the appropriate dilution by pre-test
High Background	Stop Solution contaminated	Change Stop Solution
	Color development time is too long	Control color development time
	Incorrect dilution of Biotin-antibody or Streptavidin-HRP	Follow the dilution method
	Insufficient washing	Wash sufficiently and add sufficient Wash Buffer

References:

1.Tang L, Han X (March 2013).Biomedicine Pharmacotherapy. 67 (2): 179–82.

2.Josip Madunić (2018).Thrombosis and Haemostasis. 118 (12): 2020–2036.

3.Ploug M, Gårdsvoll H, Jørgensen TJ, Lønborg Hansen L, Danø K (April 2002) Biochemical Society Transactions. 30 (2): 177–83..

4.Alfano M, Sidenius N, Blasi F, Poli G (November 2003).Journal of Leukocyte Biology. 74 (5): 750–6.

5.Harbeck N, Kates RE, Gauger K, Willems A, Kiechle M, Magdolen V, Schmitt M (March 2004).Thrombosis and Haemostasis. 91 (3): 450–6.

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