Porcine uPA ELISA Kit

Porcine Urokinase plasminogen activator ELISA Kit
Catalog Number: PU0019 (96 Tests)

Compatible samples: Cell culture fluid, body fluid, tissue homogenate, serum or plasma
Store all reagents at 2-8°C

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS. READ THROUGH ALL PROCEDURES BEFORE USE.

ASSAY PRINCIPLE
This uPA ELISA is a quantitative competitive immunoassay. The microtiter plate provided is coated with an uPA-specific antibody. Standards or experimental samples are co-incubated in wells along with an uPA-HRP conjugate. uPA in standards or samples competes with uPA-HRP conjugate for binding to the plate bound antibody. Higher levels of uPA from standards or samples leads to decreased uPA-HRP conjugate binding and reduced signal.
Captured uPA-HRP is quantitatively detected by incubation with HRP substrates (solutions A and B). Binding of the uPA-HRP is visualized by production of colorimetric reaction products that can be quantitatively measured by absorbance at 450nm.

MATERIALS
All reagents must be stored at 2-8° C. Refer to the expiration date on the label.

1	Microplate	96 strip wells	
2	Standard A	0 pg/ml	1 mL
3	Standard B	50 pg/ml	1 mL
4	Standard C	100 pg/ml	1 mL
5	Standard D	250 pg/ml	1 mL
6	Standard E	500 pg/ml	1 mL
7	Standard F	1000 pg/ml	1 mL
8	Substrate A	1 vial	6.0 mL
9	Substrate B	1 vial	6.0 mL
10	Stop Solution	1 vial	6.0 mL
11	Enzyme Solution	1 vial	6.0 mL
12	Balance Solution	1 vial	1 mL
13	Wash Solution (25×)	1 vial	50 mL

GENERAL CONSIDERATIONS
1. Samples should be handled following standard practices to minimize degradation or denaturation. Avoid multiple freeze-thaw cycles or high temperatures. For long term storage, maintain samples at temperatures that minimize degradation or denaturation.
2. Without prior knowledge of the analyte concentration, determining the amount of sample required for robust detection is difficult. Therefore, we recommend that enough sample be collected to allow for multiple dilutions to be assayed.
3. Experimental variation between wells is to be expected. We recommend assays be performed using at least two wells for every sample or standard. Readings of duplicate wells should be averaged.
4. Two blank wells containing sample buffer should be included to determine background.

REAGENT PREPARATION
1. Bring all kit components and samples to room temperature before use.
2. Bring microtiter plate to room temperature before opening. Remove the desired number of well strips and immediately reseal and store at 2-8°C.
3. Sample Preparation
   • For **cell culture supernatant, body fluid, or tissue homogenate**: add 10 µL of BALANCE SOLUTION per 100 µL of sample. If dilution is required, dilute the sample first, then add 10 µL of BALANCE SOLUTION per 100 µL of the diluted sample.
   • For **serum or blood samples**: In most cases, no dilution is necessary. Do not add BALANCE SOLUTION. Proceed directly to Step 4.
   • For **serum or blood samples (requiring dilution)**: If dilution is required in special cases (e.g., extremely high analyte levels), dilute the sample with 1× PBS or 0.9% saline. Then add 10 µL of BALANCE SOLUTION per 100 µL of diluted sample and proceed to Step 4.

If you have any questions regarding sample preparation, please contact our technical support team.

4. Dilute 40 mL of WASH SOLUTION concentrate (25×) with 960 mL of deionized or distilled water. If crystals have formed in the concentrate warm to room temperature and mix to dissolve.

ASSAY PROCEDURE
1. Add 100 µL of SAMPLE or STANDARD to the appropriate number of wells in the supplied microtiter plate. Note that wells have been pre-blocked and no additional blocking steps are required. Add 100µL of PBS (pH 7.0-7.2) or water to the blank well.
2. Add 50µL of Enzyme Solution to each well (**but NOT blank well**) in the supplied Plate and mix well.
3. Cover and incubate 1 hour at 37°C in a humid chamber.
4 Wash each well 5 times with 300-400 µL 1X WASH SOLUTION per well. After the last wash invert the plate and blot dry by tapping on absorbent paper. Note: Hold the sides of the plate frame firmly when washing to assure that all strips remain securely in the frame. Complete removal of the liquid at each step is essential for good performance.
5. Add 50 µL SUBSTRATE A to each well followed by addition of 50 µL SUBSTRATE B. Cover and incubate 10-15 minutes at room temperature. SUBSTRATE is light sensitive. Keep out of direct sunlight or cover with foil.
6. Add 50 µL of STOP SOLUTION to each well and mix well.
7. Immediately read the optical density (O.D.) at 450 nm.
8. Subtract the mean blank value from each SAMPLE or STANDARD value and calculate the mean for duplicate (or greater) wells.
9. Construct the standard curve using graph paper or statistical software.

REAGENT PREPARATION
1. The sensitivity of this assay should be approximately 1.0pg/ml.
2. This assay has high sensitivity and excellent specificity for the detection of uPA. No significant cross-reactivity or interference between uPA and any homologous proteins assayed has been observed. Species cross-reactivity has not been specifically determined.

REAGENT PREPARATION
1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Samples should be collected in pyrogen/endotoxin-free tubes.
3. When possible, avoid use of hemolyzed or lipemic sera. Centrifuge or filter samples if particulate matter is present.
4. It is recommended that all standards, controls, and samples be run at least in duplicate.
5. To ensure equal incubation times maintain a consistent order of addition from well-to-well when pipetting reagents.
6. Cover or cap all reagents when not in use.
7. Do not mix or interchange different reagent lots from various kit lots.
8. Do not use reagents after the kit expiration date.
9. Determine absorbance within 2 hours of assay completion.
10. The provided controls should be run with every assay.
11. Substrate B is light sensitive. Avoid prolonged exposure to light. Substrate B will discolor metals so contact should be avoided.
12. Incomplete washing will adversely affect the test outcome. All washing must be performed with the provided Wash Solution.
13. Washing can be performed using a squirt bottle and filling all wells to the top.
14. Do not mix reagents from different lots. It is recommended that assays be performed at least in duplicate. Standards and samples must be assayed at the same time.

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SAFETY NOTES

1. This kit contains 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) in Substrate B. TMB, present at levels greater than or equal to 0.1% is NOT identified as a carcinogen or potential carcinogen by OSHA. TMB may cause irritation to skin and eyes. Please wear appropriate personal protective equipment, including gloves, safety glasses, and lab coats when handling.
2. The Stop Solution provided in the kit is an acidic solution. Please wear appropriate personal protective equipment (gloves, safety glasses, lab coat) when handling this and all kit components.
3. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
4. All waste must be disposed of in accordance with all applicable local, state, and federal regulations.

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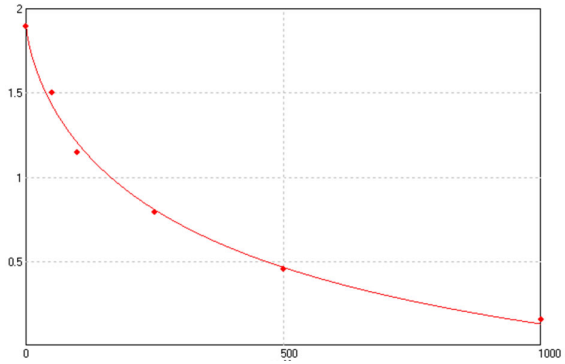
Calculation of competitive ELISA results

After obtaining raw data from the ELISA reader, the ELISA results are ready for statistical analysis. We suggest using an ELISA data analysis software for the analysis, such as CurveExpert 1.4 or GraphPad Prism. Microsoft Excel can also be a useful tool to analyze the data.

Below is an example we use CurveExpert 1.4 to process the raw data.

1. Enter ELISA Data Into Software
   Enter the standard concentration in the x-axis column and the corresponding OD values in the y-axis column.
2. Select The Best Fitting Curve
   Our lab and most companies generally recommend using a 4-parameter algorithm for the best standard curve fit. Users are welcome to try other models for calculation. The ideal curve to be picked should rise smoothly and closely resemble a straight line and the equation should be with the higher R value.
3. Calculate Target Protein Concentration
   The calculation can be performed in the software. If the samples were diluted before the ELISA, make sure to multiply the computed sample concentrations by the sample dilution factor.



Example: Standard Curve by 4-parameter algorithm

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ELISA Kits (>10,000):
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Subcategories: Cytokine, Globulins, CD & AM, Innate Immune, Immunodulator, Transplantation, Autoimmunity, Apoptosis, Tumor Immune, Hypersensitivity, Immunity, Infectious Disease, Hematology, Vascular, Signal Transduction, Neuroscience, Eye & Ear, Respiratory, Digestive, Urinary System, Aristogenesis, Endocrine, Metabolism, Enzyme, Kinase, Hormone, etc.

Proteins and Antibodies (>5,000):
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Technical Services

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NeoScientific is your premier partner in ELISA. We can make various high-quality ELISA Kits for you based on your request. Our ELISA assays are designed for minimal manipulation and rapid use. This service from NeoScientific allows you to focus on your research rather than of the arduous work of designing and building ELISA assays.

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Antibodies are critical research reagents. NeoScientific has extensive experience generating high quality monoclonal and polyclonal antisera to native and recombinant proteins as well as synthetic peptides. Using our reliable and tested immunization protocols we have had great success generating high-titer, high-affinity custom antibodies against a diverse set of antigens from numerous species.

Peptide Synthesis:
NeoPeptide offers a comprehensive range of Peptide Synthesis Services from peptide sequence design advice to peptide modifications. Our emphasis is on providing high quality peptides at a competitive price. All peptides synthesized are provided with a complete quality control package, which includes HPLC chromatograms to ensure purity and MS analysis to confirm identity.

Custom Protein Expression and Purification:
NeoScientific's custom recombinant protein services provide a comprehensive portfolio of protein expression systems, including bacteria, yeast, baculovirus infected insect cells, and mammalian cells. With our proprietary optimization technology, the expression level can be significantly increased. We also offer a full spectrum of protein purification methods to achieve the ideal level of protein purity. We work in partnership with our clients to ensure efficient and high-quality reagent protein production with customized solutions, flexible production scale, and proprietary technologies. We can also provide process development and scale-up production of biologics for research purposes.

Innovative Technolog:

GlycoPep™: Peptide with PTM
We offer our customers a novel technology platform for development of Post Translationally Modified (PTM) peptides for diagnostics, epitope mapping, companion drug biomarkers, and monitoring immune signatures. Our patent portfolio covers enzymes & methods for production of these glycosylated PTM-peptides and biomarkers. We can provide our customers with custom made glycosylated peptides to meet their research needs.

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We utilize a novel technology to display 6 peptides specific to your protein of interest on a highly immunogenic vector. This system has been used in over thousands projects delivering high quality monoclonal antibodies at the lowest cost available.