LMA140Po 96 Tests

Magnetic Luminex Assay Kit for (uPA)

Plasminogen Activator, Urokinase

Organism Species: Sus scrofa; Porcine (Pig)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Version 1.0

[INTENDED USE]

The kit is a magnetic luminex assay kit applies sandwich enzyme immunoassay for the in vitro quantitative measurement of uPA in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids. The kit can be used to detect porcine samples.

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
96-well plate	1	Plate sealer for 96 wells	4
Pre-Mixed Standard	2	Standard Diluent	1×20mL
Pre-Mixed Magnetic beads	1	Analysis buffer	1×20mL
Pre-Mixed Detection Reagent A	1×120µL	Assay Diluent A	1×12mL
Detection Reagent B (PE-SA)	1×120µL	Assay Diluent B	1×12mL
Sheath Fluid	1×10mL	Wash Buffer (30 × concentrate)	1×20mL
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[MATERIALS REQUIRED BUT NOT SUPPLIED]

1. Luminex MAGPIX®, Luminex 100™, Luminex 200™, or Bio-Rad®, Bio-Plex® analyzer (It is recommended that the instrument be preheated, self-checked and calibrated before use).
2. Single or multi-channel pipettes with high precision and disposable tips.
3. Microcentrifuge Tubes.
4. Deionized or distilled water.
5. Magnetic frame.
6. Container for Wash Solution.
7. 0.01mol/L (or 1×) Phosphate Buffered Saline(PBS), pH7.0-7.2.
8. Vortex oscillator.
9. Incubated Shaker.

[STORAGE OF THE KITS]

1. **For unused kit:** All the reagents should be kept according to the labels on vials. The **Standard, Detection Reagent A, Detection Reagent B** and the **96-well strip plate** should be stored at -20°C upon receipt while the others should be at 4°C.
2. **For used kit:** When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.

Note:

It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box. All components are stable up to the expiration date.

[SAMPLE COLLECTION AND STORAGE]

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (catalog: IS007, different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10,000×g. Collect the supernates and assay immediately or aliquot and store at ≤-20°C.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

[Luminex200 analyzer]

1. Analytes volume:50µL.
2. Bead type:MagPlex.
3. Total Count:50 count/region.
4. Collect Median Fluorescence Intensity (MFI).

[REAGENT PREPARATION]

1. Bring all kit components and samples to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. **Standard** - Reconstitute the **Standard** with 1.0mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 10.0ng/mL. Please prepare 6 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. (SAMPLES. Please always refer to the hard copy manual included in the kit for your experiment.)

item	1	2	3	4	5	6	7	Tube
uPA	10.0	2.5	0.62	0.16	0.04	0.01	0	ng/mL

3. **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A** and **B**, respectively..
4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for one pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

[SAMPLE PREPARATION]

1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Sample should be diluted by PBS.
3. If the volume of samples are limited, please dilute the samples with standard diluent to appropriate volume.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
6. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
7. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernates may not be detected by the kit.
8. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

[ASSAY PROCEDURE]

1. Add 200µL analysis buffer solution to each well of the plate for pre-wetting. Shake the plate at room temperature for 10 minutes on shaker. Then, remove the liquid in the well for later use.
2. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100µL each of dilutions of standard (follow Reagent Preparation step 2), blank and samples into the appropriate wells,add 10µL magnetic beads in each well,then vortex the pre-mixed magnetic beads. Cover with the Plate sealer. Incubate for 90min at 37°C on shaker with speed of 800rpm, amplitude of 2-4 mm, to avoid settling of magnetic beads. (If the volume of samples are limited, please add Standard Diluent together with the samples to 100ul/well.)
3. Put the plate on magnetic frame for 2min, remove the liquid of each well with plate on the magnetic frame, don’t wash.
4. Add 100µL of **Detection Reagent A** working solution to each well, cover the wells with the plate sealer and incubate for 60min at 37°C on shaker with speed of 800rpm, amplitude of 2-4 mm.
5. Put the plate on magnetic frame for 2min, remove the liquid of each well. Add 200µL of 1× Wash Solution to each well and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper. This process could be performed by magnetic plate autowasher.
6. Remove the magnetic frame, add 100µL of **Detection Reagent B** working solution to each well, cover the wells with the plate sealer and incubate for 30min at 37°C on shaker with speed of 800rpm, amplitude of 2-4 mm.
7. Put the plate on magnetic frame for 2min, remove the liquid of each well. Add 200µL of 1× Wash Solution to each well and let it sit for 1~2 minutes. Totally wash 3 times as conducted in step 5.
8. Remove the magnetic frame, add sheath fluid 100µL to each well, swirl for 2 min, make the Magnetic beads in suspension, then run the machine and conduct the reading. Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker with speed of 800rpm, amplitude of 2-4 mm.

Note:

1. **Samples or reagents addition: Please use the freshly prepared Standard.** Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
2. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled.
3. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
4. If the humidity in the laboratory is below 60%, it is recommended to use a humidifier to increase the humidity.

[TEST PRINCIPLE]

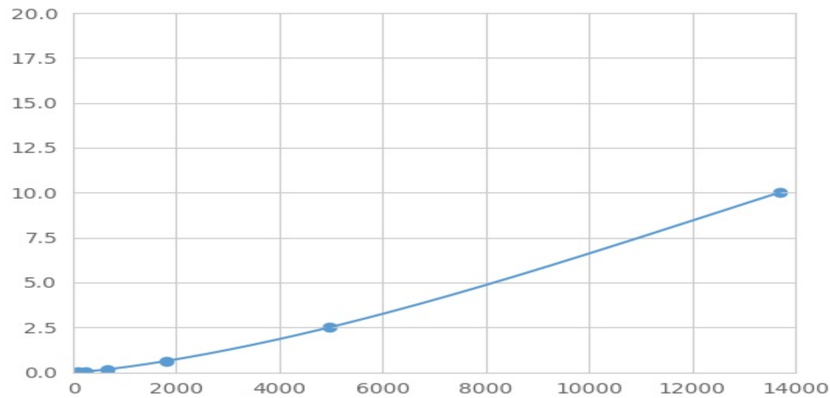
uPA antibody was coated on the surface of magnetic beads, to make solid phase carrier. Standard or sample were added to the magnetic beads, respectively, in which uPA was bound to the antibody connected to the solid phase carrier, and then biotinylated uPA antibody was added. After the unbound biotinylated antibody was washed, PE-labeled avidin was added. There is a positive correlation between the concentration of uPA in the sample and the value of MFI (Median Fluorescence Intensity). Luminex analyzer was used to determine and calculate the sample concentration.

[CALCULATION OF RESULTS]

Median MFI value of each standard, control, and samples subtract the zero standard MFI value. Create standard curves with uPA concentration on the Y-axis and MFI on the X-axis, respectively. Draw a best fit curve through the points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

[TYPICAL DATA]

In order to make the calculation easier, we plot the MFI value of the standard as the horizontal axis (X-axis) against the concentration of the standard as the Y-axis, although the concentration is the independent variable and the MFI value is the dependent variable. In order to make the experimental results intuitive, the original data rather than logarithm values are provided in the figure. Logarithmic values are recommended for standard graphs. However, the MFI of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), plotting log of the data to establish standard curve for each test is recommended. Typical standard curve below is provided for reference only.



Typical Standard Curve for uPA Magnetic Luminex Assay.

[DETECTION RANGE]

uPA: 0.01-10ng/mL. The standard curve concentrations used were 10.0ng/mL, 2.5ng/mL, 0.62ng/mL, 0.16ng/mL, 0.04ng/mL, 0.01ng/mL.

[SENSITIVITY]

The minimum detectable dose of uPA is typically less than None.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by subtracting two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

[SPECIFICITY]

This assay has high sensitivity and excellent specificity for detection of uPA.

No significant cross-reactivity or interference between uPA and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between the targets and all the analogues, therefore, cross reaction may still exist.

[STABILITY]

The stability of Luminex kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

[ASSAY PROCEDURE SUMMARY]

1. Preparation of standards, reagents and samples before the experiment;
2. Add 100µL standard or sample to each well,
   add 10µL magnetic beads, and incubate 90min at 37°C on shaker;
3. Remove liquid on magnetic frame, add 100µL prepared Detection Reagent A. Incubate 60min at 37°C on shaker;

4. Wash plate on magnetic frame for three times;
5. Add 100µL prepared Detection Reagent B, and incubate 30 min at 37°C on shaker;
6. Wash plate on magnetic frame for three times;
7. Add 100µL sheath solution, swirl for 2 minutes, read on the machine.

[IMPORTANT NOTE]

1. Limited by the current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, so the kit should be used prior to the expiration date. And please store the kits exactly according to the instruction.
3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website is only for reference.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
6. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microplate from the storage bag until needed.
7. Variation in sample preparation and each step of experimental operation may cause different results. In order to get better reproducible results, the operation of each step in the assay should be controlled.
8. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
9. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.
10. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
11. Please predict the concentration of target molecules in samples, or arrange a preliminary experiment, it is a good way to solve specific problem, e.g. the concentration of samples are beyond the detection range of the kit.
12. The kit might not be suitable for detection of samples from some special experiment, for instance, knock-out experiments, due to their uncertainty of effectiveness.
13. The instruction manual is also for the kit of 48T, but all reagents of 48T kit are reduced by half.
14. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.

[TROUBLE SHOOTING]

Problem	Possible Source	Correction Action

Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low MFI	Inadequate reagent volumes added to wells	Calibrate pipettes and Add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate reagent failure	Replace the conjuate reagent
	Incorrect Conjugate reagent dilution	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Use new sample and repeat assay