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ELISA Kit for Bovine Plasminogen activator,Urokinase Receptor (uPAR;CD87)

Instruction manual
Cat. No.: E0141Bo
Size: 96 tests
FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES

INTENDED USE

The ELISA kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of bovine uPAR in serum, plasma, cell culture supernates and other biological fluids.

REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1
Standard (freeze dried)	2
Standard Diluent	1 × 20ml
Detection Reagent A	1 × 120μl
Detection Reagent B	1 × 120μl
Assay Diluent A (2 x concentrate)	1 × 6ml
Assay Diluent B(2 x concentrate)	1 × 6ml
TMB Substrate	1 × 9ml
Stop Solution	1 × 6ml
Wash Buffer(30 x concentrate)	1 × 20ml
Plate sealer for 96 wells	4
Instruction manual	1

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader with 450 ± 10 nm filter.
2. Precision single and multi-channel pipettes and pipette tips with disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.

6. Container for Wash Solution

STORAGE OF THE KITS

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℃ upon being received. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

TEST PRINCIPLE

The microtiter plate provided in this kit has been pre-coated with an antibody specific to uPAR. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for uPAR. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain uPAR, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm±2 nm. The concentration of uPAR in the samples is then determined by comparing the O.D. of the samples to the standard curve.

SUMMARY AND EXPLANATION

The Urokinase receptor, also known as uPA receptor or uPAR or CD87 (Cluster of Differentiation 87), is multidomain glycoprotein tethered to the cell membrane with a glycosylphosphotidylinositol (GPI) anchor. uPAR was originally identified as a saturable binding site for urokinase on the cell surface.

uPAR consists of three different domains of the Ly-6/uPAR/alpha-neurotoxin family. All three domains are necessary for high affinity binding of the primary ligand, urokinase. It has been possible to express uPAR recombinantly in CHO-cells and S2 cells from Drosophila melanogaster. 4 out of 5 of the possible glycosylation sites are used in vivo giving the protein a molecular weight of 50-60 kDA. Recently the structure of uPAR was solved by X-ray crystallography in complex with an peptide antagonist and with its native ligand, urokinase.

Besides the primary ligand urokinase, uPAR interacts with several other proteins, among others: vitronectin, the uPAR associated protein (uPARAP) and the integrin family of membrane proteins.

uPAR is a part of the plasminogen activation system, which in the healthy body is involved in tissue reorganization events such as mammary gland involution and wound healing. In order to be able to reorganize tissue it is important, that the old tissue can be degraded. An important mechanism in this degradation is the proteolysis cascade initiated by the plasminogen activation system. uPAR binds urokinase and thus restricts plasminogen activation to the immediate vicinity of the cell membrane. Thus uPAR seems to be an important player in the regulation of this process.

However the components of the plasminogen activation system have been found to be highly expressed in many malignant tumors, indicating that tumors are able to hijack the system, and use it in metastasis. Thus inhibitors of the various components of the plasminogen activation system has been sought as possible anticancer drugs.
uPAR has been involved in various other non-proteolytical processes related to cancer, such as cell migration, cell cycle regulation and cell adhesion.

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℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Remove serum and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

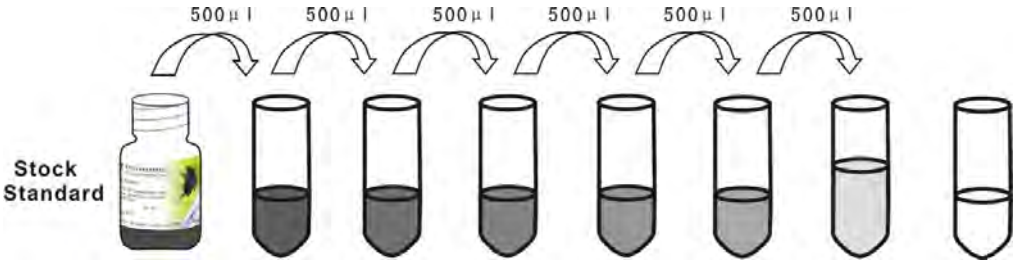
Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must stored at -20℃ (≤1 month) or -80℃ (≤ 2 months) to avoid loss of bioactivity and contamination.
2. When performing the assay slowly bring samples to room temperature.
3. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature(18-25℃) before use.
2. **Standard -** Reconstitute the **Standard** with 1.0 ml of **Standard Diluent,** kept for 10 minutes at room tempreture, shake gently(not to foam). The concentration of the standard in the stock solution is 8,000 pg/mL. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 8,000 pg/mL, 4,000 pg/mL, 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, and the last EP tubes with **Standard Diluent** is the blank as 0 pg/mL.



Tube	1	2	3	4	5	6	7	8
pg/mL	8,000	4,000	2,000	1,000	500	250	125	0

3. **Assay Diluent A and Assay Diluent B -** Dilute 6mL of Assay Diluent A or B Concentrate(2×) with 6ml of deionized or distilled water to prepare 12 mL of Assay Diluent A or B. **The prepared working dilution can't be frozen.**
4. **Detection Reagent A and Detection Reagent B -** Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with working **Assay Diluent A or B,** respectively (1:100).
5. **Wash Solution** - Dilute 20mL of Wash Solution Concentrate(30×) with 580ml of deionized or distilled water to prepare 600 mL of Wash Solution(1×).
6. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37℃ directly.
2. Making serial dilution in the wells directly is not permitted.
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 have 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 once 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 have completely dissolved.

ASSAY PROCEDURE

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.

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μl each of dilutions of standard (see Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 2 hours at 37℃.
2. Remove the liquid of each well, don't wash.
3. Add 100 μl of **Detection Reagent A** working solution to each well. Incubate for 1

hour at 37℃ after covering it with the Plate sealer.

4. Aspirate the solution and wash with 400 μl of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100 μl of **Detection Reagent B** working solution to each well. Incubate for 30 minutes at 37℃ after covering it with the Plate sealer.
6. Repeat the aspiration/wash process for five times as conducted in step 4.
7. Add 90 μl of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 15 - 25 minutes at 37℃ (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50 μl of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

Note:

1. **Assay preparation:** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4℃ until the kits expiry date.
2. **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 as possible. 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 each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **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 have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential to 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 falsely elevated absorbance reading.
5. **Controlling of reaction time:** Observe the change of colour after adding **TMB Substrate** (e.g. observation once every 10 minutes)**,** if the colour is too deep, add

Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. **TMB Substrate** is easily contaminated. Please protect it from light.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the uPAR concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

DETECTION RANGE

125-8,000 pg/mL. The standard curve concentrations used for the ELISA's were 8,000 pg/mL, 4,000 pg/mL, 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL.

SENSITIVITY

The minimum detectable dose of bovine uPAR is typically less than 32 pg/mL.

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 the mean O.D. Value of 20 replicates of the zero standard added by their three standard deviations.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of bovine uPAR. No significant cross-reactivity or interference was observed.

IMPORTANT NOTE

1. Limited by the current condition 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, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.
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 microtiter plate from the storage bag until needed.
7. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450nm wavelength is acceptable for use in absorbance measurement.
8. Valid period: six months.
9. The instruction manual also suit for the kit of 48T, but all reagents of 48T kit is reduced by half.

PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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