PLAUR (Human) ELISA Kit

Catalog Number KA0426

96 assays

Version: 04

Intended for research use only

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Table of Contents

Introduction

Intended Use

For quantitive detection of human uPAR in serum, plasma, body fluids, tissue lysates and cell culture supernates.

Background

The urokinase-type plasminogen activator receptor (uPAR) is a key molecule in the regulation of cell-surface plasminogen activation and, as such, plays an important role in many normal as well as pathological processes.[1] The cDNA for Mo3, an activation antigen expressed by human monocytes and myelomonocytic cell lines after stimulation by a variety of agents. Mo3 expression in vivo is associated predominantly with macrophages in inflammatory sites. It is a highly glycosylated protein of about 50 kD in monocytes where it is anchored to the plasma membrane by glycosyl-phosphatidylinositol linkage. The complete coding sequence of the cDNA has been found to encode 335 amino acids including a predicted signal peptide of 22 residues and a hydrophobic C-terminal portion. Mo3 is identical to the human receptor for the urokinase plasminogen activator.[2] UPAR is a useful prognostic marker for biologically aggressive forms of endometrial cancer.[3] PLAUR is located at chromosome 19q13.1-q13.2.[1] The standard product used in this kit is recombinant human uPAR, consisting of 287 amino acids with the molecular mass of 31KDa.

Principle of the Assay

The PLAUR (Human) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human uPAR specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human uPAR amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
One 96-well plate precoated with anti- human uPAR antibody	96 (8x12) wells
Lyophilized recombinant human uPAR standard	10 ng x 2
Biotinylated anti- human uPAR antibody, dilution 1:100	130 µl
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µl
Sample diluent buffer	30 ml
Antibody diluent buffer	12 ml
ABC diluent buffer	12 ml
TMB color developing agent	10 ml
TMB stop solution	10 ml

Storage Instruction

Store at 4℃ for 6 months, at -20℃ for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).

  Preparation of 0.01M TBS:

  Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

  Preparation of 0.01 M PBS:

  Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Precautions for Use

- ✓ To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using Kit, spin tubes and bring down all components to bottom of tube.
- ✓ Duplicate well assay was recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ To avoid to use the reagents from different batches together.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃ for 30 min before using.

Assay Protocol

Reagent Preparation

- Reconstitution of the human uPAR standard: uPAR standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of uPAR standard (10ng per tube) are included in each kit. Use one tube for each experiment.
  - ✓ 10,000pg/ml of human uPAR standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
  - ✓ 4000pg/ml of human uPAR standard solution: Add 0.4 ml of the above 10ng/ml uPAR standard solution into 0.6 ml sample diluent buffer and mix thoroughly.
  - ✓ 2000pg/ml→62.5pg/ml of human uPAR standard solutions: Label 6 Eppendorf tubes with 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 4000pg/ml uPAR standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

  Note: The standard solutions are best used within 2 hours. The 10ng/ml standard solution may be stored at 4℃ for up to 12 hours, or at -20℃ for up to 48 hours. Avoid repeated freeze-thaw cycles.

- Preparation of biotinylated anti-human uPAR antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
  - ✓ The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  - ✓ Biotinylated anti-human uPAR antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µl Botinylated anti-human uPAR antibody to 99 µl antibody diluent buffer.)

- Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
  - ✓ The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  - ✓ Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1 µl ABC to 99 µl ABC diluent buffer.)

Sample Preparation

Store samples to be assayed within 24 hours at 2-8℃. For long-term storage, aliquot and freeze samples at -20℃. Avoid repeated freeze-thaw cycles.

- Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20℃
- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20℃.
- Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20℃. Citrate is not recommended as the anticoagulant.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- High target protein concentration (40-400ng/ml). The working dilution is 1:100. i.e. Add 3 µl sample into 297 µl sample diluent buffer.
- Medium target protein concentration (4-40ng/ml). The working dilution is 1:10. i.e. Add 25 µl sample into 225 µl sample diluent buffer.
- Low target protein concentration (62.5-4000pg/ml). The working dilution is 1:2. i.e. Add 100 µl sample to 100 µl sample diluent buffer.
- Very Low target protein concentration (≤62.5pg/ml). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37℃ for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard uPAR detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of uPAR amount in samples.

1. Aliquot 0.1ml per well of the 4000pg/ml, 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml human uPAR standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human serum, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “Sample Dilution Guideline” above for details. We recommend that each human uPAR standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37℃ for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human uPAR antibody working solution into each well and incubate the

plate at 37℃ for 60 min.

5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

   (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. *Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)*
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37℃ for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (see Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37℃ in dark for 20-25 min (*Note: For reference only, the optimal incubation should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human uPAR standard solutions; the other wells show no obvious color*).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

Summary

1. Add samples and standards and incubate the plate at 37℃ for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37℃ for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37℃ for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37℃ in dark for 20-25 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human uPAR concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical result

Typical Data Obtained from human uPAR

Concentration (pg/ml)	0.0	62.5	125	250	500	1000	2000	4000
O.D.	0.004	0.042	0.090	0.178	0.384	0.744	1.505	2.317

(TMB reaction incubate at 37℃ for 20 min)

Performance Characteristics

- Range
  62.5pg/ml-4000pg/ml

- Sensitivity
  < 4pg/ml

- Specificity
  Nature and recombinant human uPAR.
- Cross-reactivity
  No detectable cross-reactivity with any other cytokine.

Resources

References

1. Borglum, A. D.; Byskov, A.; Ragno, P.; Roldan, A. L.; Tripputi, P.; Cassani, G.; Dano, K.; Blasi, F.; Bolund, L.; Kruse, T. A. Assignment of the urokinase-type plasminogen activator receptor gene (PLAUR) to chromosome 19q13.1-q13.2. Am. J. Hum. Genet. 50: 492-497, 1992.
2. Min, H. Y.; Semnani, R.; Mizukami, I. F.; Watt, K.; Todd, R. F., III; Liu, D. Y. cDNA for Mo3, a monocyte activation antigen, encodes the human receptor for urokinase plasminogen activator. J. Immun. 148: 3636-3642, 1992.
3. Memarzadeh, S.; Kozak, K. R.; Chang, L.; Natarajan, S.; Shintaku, P.; Reddy, S. T.; Farias-Eisner, R. Urokinase plasminogen activator receptor: prognostic biomarker for endometrial cancer. Proc. Nat. Acad. Sci. 99: 10647-10652, 2002. Note: Erratum: Proc. Nat. Acad. Sci. 99: 12501 only, 2002.

Plate Layout

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