Product Information Sheet

Human uPA ELISA Kit

Catalog No. EK0535

Size 96T

Range 62.5pg/ml-4000pg/ml

Sensitivity < 5 pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Storage

Store at 4℃ for frequent use, at -20℃ for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration

Four months at 4℃ and eight months at -20℃.

Application

For quantitative detection of human uPA in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Human uPA ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human uPA 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 uPA amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human uPA standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human uPA antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human uPA antibody : 130μl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130μl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. 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

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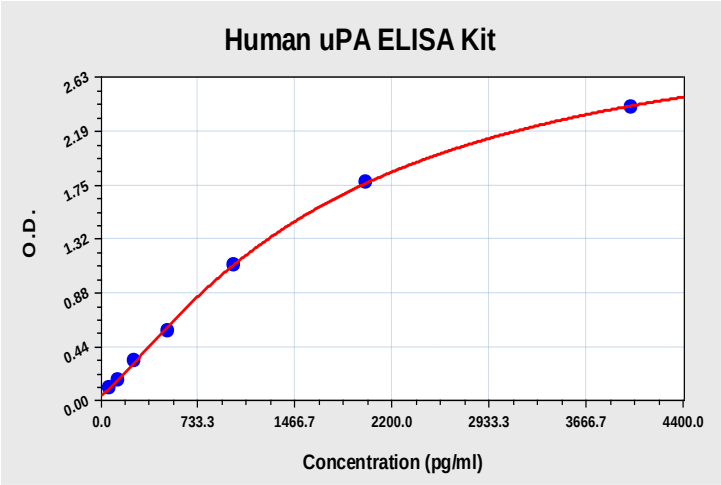
and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. 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.

Human uPA ELISA Kit-1X96 Well Plate Image



Background

Plasminogen activator,urokinase (PLAU, uPA) converts plasminogen to plasmin. Plasmin is involved in processing of amyloid precursor protein and degrades secreted and aggregated amyloid-beta, a hallmark of Alzheimer disease (AD).[1] Urokinase has a molecular mass of about 54 kD and is composed of 2 disulfide-linked chains, A and B, of molecular masses 18 kD and 33 kD, respectively. It localized on 10q24. uPA facilitates cell migration by localizing proteolisys on the cell surface and by inducing intracellular signalling pathways. In human vascular smooth muscle cell (VSMC), uPA stimulates migration via the uPA receptor (uPAR) signalling complex containing TYK2 and phosphatidylinositol 3-kinase (PI3-K).[2]

Reference

1. Finckh, U.; van Hadeln, K.; Muller-Thomsen, T.; Alberici, A.; Binetti, G.; Hock, C.; Nitsch, R. M.; Stoppe, G.; Reiss, J.; Gal, A. : Association of late-onset Alzheimer disease with a genotype of PLAU, the gene encoding urokinase-type plasminogen activator on chromosome 10q22.2. *Neurogenetics* 4: 213-217, 2003.
2. Kiian, I.; Tkachuk, N.; Haller, H.; Dumler, I. : Urokinase-induced migration of human vascular smooth muscle cells requires coupling of the small GTPase RhoA and Rac1 to the Tyk2/PI3-K signalling pathway. *Thromb. Haemost.* 89: 904-914, 2003.