Product Information Sheet

Mouse Cystatin C ELISA Kit

Catalog No. EK0679

Size 96T

Range 0.312ng/ml-20ng/ml

Sensitivity < 10 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 mouse Cystatin C in sera, plasma, body fluids, tissue lysates or cell culture supernates.

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Principle

Mouse Cystatin C ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse Cystatin C specific-specific polyclonal antibodies were precoated onto 96-well plates. The mouse 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 mouse Cystatin C amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant mouse Cystatin C standard: 20ng/tube×2.
2. One 96-well plate precoated with anti- mouse Cystatin C antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- mouse Cystatin C 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.

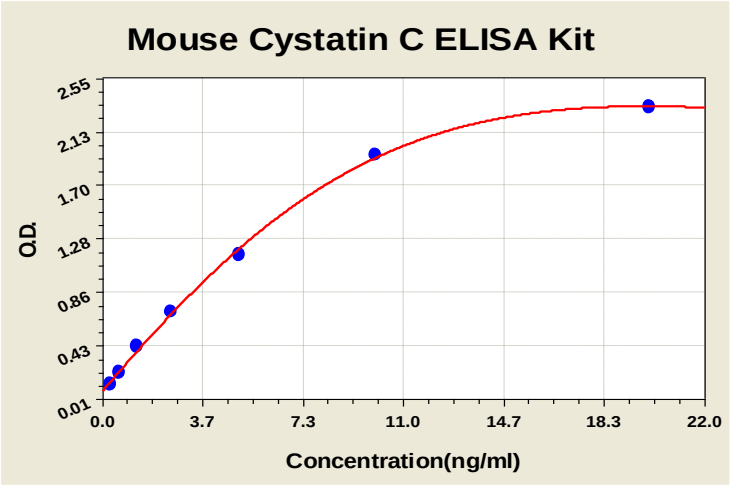
   Preparation of 0.01 M **PBS:** Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6.

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.

Mouse Cystatin C ELISA Kit-1X96 Well Plate Image



Background

Cystatin C or cystatin 3 (formerly gamma trace, post-gamma-globulin or neuroendocrine basic polypeptide), a protein encoded by the CST3 gene, was originally described as a constituent of normal cerebrospinal fluid (CSF) and of urine from patients with renal failure.[1] Cystatin 3 has a low molecular weight (approximately 13.3 kilodaltons), and it is removed from the bloodstream by glomerular filtration in the kidneys. In mouses, all cells with a nucleus (cell core containing the DNA) produce cystatin C as a chain of 120 amino acids. It is found in virtually all tissues and bodily fluids. Cystatin C, which belongs to the type II cystatin gene family, is a potent inhibitor of lysosomal proteinases[2] (enzymes from a special subunit of the cell that break down proteins) and probably one of the most important extracellular inhibitors of cysteine proteases[3] (it prevents the breakdown of proteins outside the cell by a specific type of protein degrading enzymes). Moreover, cystatin C is involved in network reorganization in the epileptic dentate gyrus.[4] The standard product used in this kit is recombinant mouse Cystatin C, consisting of 120 amino acids with the molecular mass of 15KDa.

Reference

1. Grubb, A.; Lofberg, H. : Mouse gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. *Proc. Nat. Acad. Sci.* 79: 3024-3027, 1982.
2. Pirttila, T. J.; Manninen, A.; Jutila, L.; Nissinen, J.; Kalviainen, R.; Vapalahti, M.; Immonen, A.; Paljarvi, L.; Karkola, K.; Alafuzoff, I.; Mervaala, E.; Pitkanen, A. : Cystatin C expression is associated with granule cell dispersion in epilepsy. *Ann. Neurol.* 58: 211-223, 2005.
3. Abrahamson, M. : Mouse cysteine proteinase inhibitors: isolation, physiological importance, inhibitory mechanism, gene structure and relation to hereditary cerebral hemorrhage. *Scand. J. Clin. Lab. Invest.* 48 (suppl. 191): 21-31, 1988.
4. Pirttila, T. J.; Manninen, A.; Jutila, L.; Nissinen, J.; Kalviainen, R.; Vapalahti, M.; Immonen, A.; Paljarvi, L.; Karkola, K.; Alafuzoff, I.; Mervaala, E.; Pitkanen, A. : Cystatin C expression is associated with granule cell dispersion in epilepsy. *Ann. Neurol.* 58: 211-223, 2005.