



Product Information Sheet

Mouse MIP-2 ELISA Kit

Catalog No. EK0452
Size 96T
Range 15.6pg/ml-1000pg/ml
Sensitivity < 5pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Storage

Store at 4°C for frequent use, at -20°C for infrequent use.

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

Expiration

Four months at 4°C and eight months at -20°C.

Application

For quantitative detection of mouse MIP-2 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Mouse MIP-2 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse MIP-2 specific-specific monoclonal antibodies were precoated onto 96-well plates. The mouse specific detection monoclonal 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 BMP-2 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant mouse MIP-2 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- mouse MIP-2 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- mouse MIP-2 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. and Automated plate washer.
2. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended if there are a large amount of samples for detection.
3. Clean tubes and Eppendorf tubes.
4. 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 H₂O 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 Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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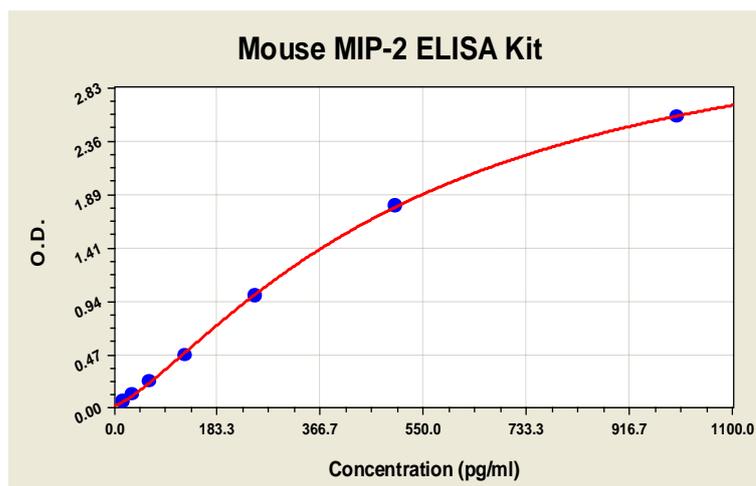
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Notice for Application of Kit

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°C for 30 min before using.

Mouse MIP-2 ELISA Kit-1X96 Well Plate Image



Background

MIP is a member of the aquaporin family of membrane-bound water channels.¹ MIP family proteins are thought to contain 6 TM domains. Sequence analysis suggests that the proteins may have arisen through tandem, intragenic duplication from an ancestral protein that contained 3 TM domains.² Major intrinsic protein (MIP, also called MP26) is the predominant fiber cell membrane protein of the ocular lens.³ The major intrinsic protein (MIP) of the vertebrate eye lens is the first identified member of a sequence-related family of cell-membrane proteins that appears to have evolved by gene duplication. Several members of the MIP family transport water (aquaporins), glycerol and other small molecules in microbial, plant and animal cells.⁴ The standard used in this kit is recombinant mouse MIP-2(A28-N100), consisting of 73 amino acids with the molecular mass of 8KDa

Reference

1. Berry, V., Francis, P., Kaushal, S., Moore, A., Bhattacharya, S. Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q. *Nature Genet.* 25: 15-17, 2000.
2. Chrispeels MJ, Agre P (1994). "Aquaporins: water channel proteins of plant and animal cells". *Trends Biochem. Sci.* 19 (10): 421-425.
3. Pisano, M. M., Chepelinsky, A. B. Genomic cloning, complete nucleotide sequence, and structure of the human gene encoding the major intrinsic protein (MIP) of the lens. *Genomics* 11: 981-990, 1991.
4. Shiels, A., Bassnett, S. Mutations in the founder of the MIP gene family underlie cataract development in the mouse. *Nature Genet.* 12: 212-215, 1996.

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