



Product Information Sheet

Human MICA ELISA Kit

Catalog No. EK0812
Size 96T
Range 62.5pg/ml-4000pg/ml
Sensitivity < 10pg/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 human MICA in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

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

Kit Components

1. Lyophilized recombinant human MICA standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human MICA antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human MICA 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 is a large amount of samples.
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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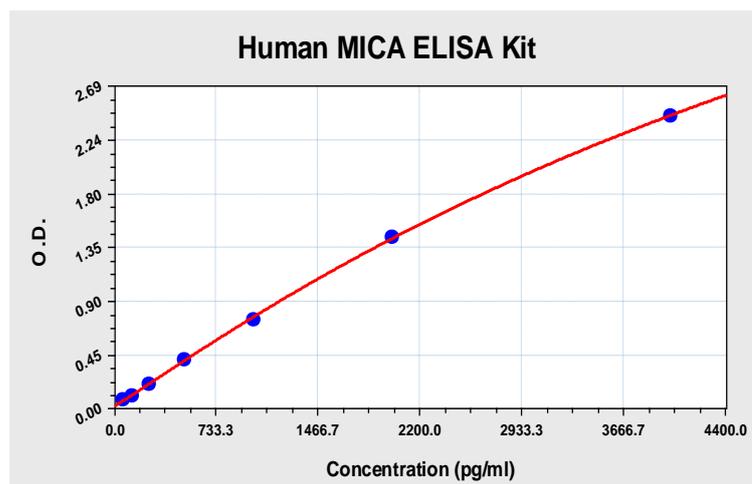
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Notice for Application of Kit

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.

Human MICA ELISA Kit-1X96 Well Plate Image



Background

MHC class I polypeptide-related sequence A is a protein that in humans is encoded by the *MICA* gene.¹ The *MICA* gene encodes a 383-amino acid polypeptide with a predicted mass of 43 kD. The *MICA* and *MICB* genes occur in a 200-kb region spanning the *TNFA* and *TNFB* cluster at 6p21.3.² *MICA* and the closely related *MICB* were recognized by intestinal epithelial T cells expressing diverse V-delta-1 gamma/delta TCRs.³ The *MICA* protein product is expressed on the cell surface, although unlike canonical class I molecules does not seem to associate with beta-2-microglobulin. It is further distinguished by its unusual exon-intron organization and preferential expression in fibroblasts and epithelial cells. It is thought that *MICA* functions as a stress-induced antigen that is broadly recognized by NK cells, NKT cells, and most of the subtypes of T cells. *MICA* and other members of this family may have been selected for specialized functions that are either ancient or derived from those of typical MHC class I genes, in analogy to some of the nonclassic mouse H-2 genes. The standard product used in this kit is recombinant *MICA*, which is composed of 286 amino acids with the molecular mass of 59KDa.

Reference

1. Bahram S, Bresnahan M, Geraghty DE, Spies T (Aug 1994). "A second lineage of mammalian major histocompatibility complex class I genes". *Proc Natl Acad Sci U S A* 91 (14): 6259-63.
2. Nalabolu, S. R.; Shukla, H.; Nallur, G.; Parimoo, S.; Weissman, S. M. : Genes in a 220-kb region spanning the TNF cluster in human MHC. *Genomics* 31: 215-222, 1996.
3. Groh, V.; Steinle, A.; Bauer, S.; Spies, T. : Recognition of stress-induced MHC molecules by intestinal epithelial gamma-delta T cells. *Science* 279: 1737-1740, 1998.

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