Anti-Human MICA Antibody [PSH11-39] - BSA and Azide free (Capture)

HA723327

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH11-39

Description: Widely expressed membrane-bound protein which acts as a ligand to stimulate an activating receptor KLRK1/NKG2D, expressed on the surface of essentially all human natural killer (NK), gammadelta T and CD8 alphabeta T-cells. Up-regulated in stressed conditions, such as viral and bacterial infections or DNA damage response, serves as signal of cellular stress, and engagement of KLRK1/NKG2D by MICA triggers NK-cells resulting in a range of immune effector functions, such as cytotoxicity and cytokine production. Widely expressed with the exception of the central nervous system where it is absent. Expressed predominantly in gastric epithelium and also in monocytes, keratinocytes, endothelial cells, fibroblasts and in the outer layer of Hassal's corpuscles within the medulla of normal thymus. In skin, expressed mainly in the keratin layers, basal cells, ducts and follicles. Also expressed in many, but not all, epithelial tumors of lung, breast, kidney, ovary, prostate and colon. In thyomas, overexpressed in cortical and medullar epithelial cells. Tumors expressing MICA display increased levels of gamma delta T-cells. A common, chronic inflammatory disease of the skin with multifactorial etiology. It is characterized by red, scaly plaques usually found on the scalp, elbows and knees. These lesions are caused by abnormal keratinocyte proliferation and infiltration of inflammatory cells into the dermis and epidermis.

Immunogen:	Recombinant protein within Human MICA aa 24-308 (HA210951).
Positive control:	Recombinant Human MICA protein (HA210951).
Subcellular location:	Cell membrane. Cytoplasm.
Database links:	SwissProt: Q29983 Human
Recommended Dilutions:	
ELISA(Cap)	Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH11-40] to Human MICA antibody (Detector) (HA723328) and Recombinant Human MICA protein (HA210951) as the standard. The reference range value is 31.2-4,000 pg/mL.
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

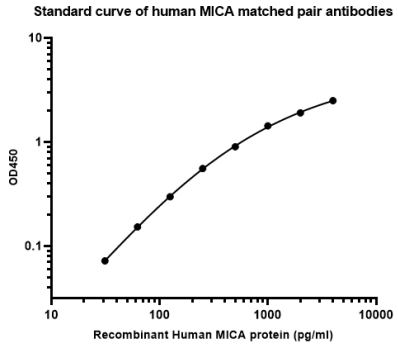

Fig1: Sandwich ELISA analysis of human MICA matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723327) diluted in carbonate/bicarbonate buffer, at a concentration of 2ug/ml overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human MICA protein (HA210951) starting from 4,000 pg/ml to 0 pg/ml and detect antibody (HA723328, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

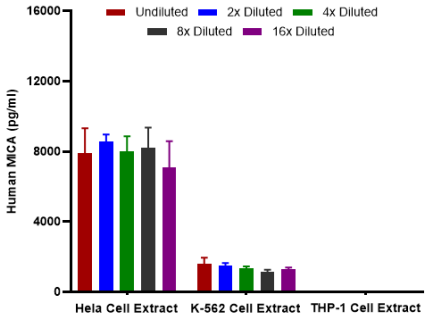

Fig2: Interpolated concentrations of native MICA in Hela, K-562 and THP-1 extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native MICA was measured in duplicate at different sample concentrations and interpolated from the MICA standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean MICA concentration was determined to be 7,949 pg/mL in Hela and 1,379 pg/mL in K-562 cell extract. There was no detectable signal in THP-1 cell extract.

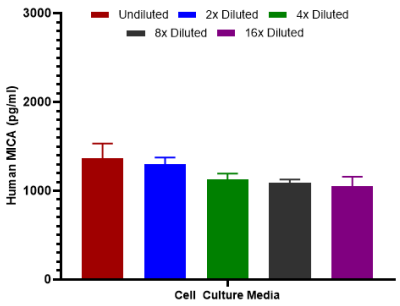

Fig3: Interpolated concentrations of spiked MICA in cell culture media samples.

The concentrations of MICA were measured in duplicates, interpolated from the MICA standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

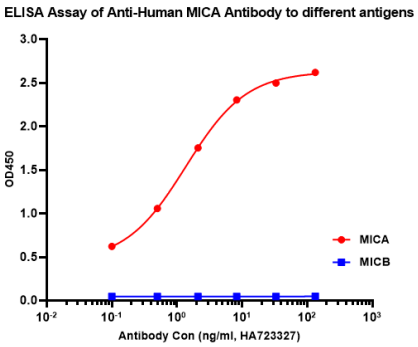

Fig4: ELISA Assay of Anti-Human MICA Antibody (HA723327) to different antigens.

Indirect ELISA analysis of Human MICA was performed by coating wells of a 96-well plate with 50 µl per well of Human MICA antigen and Human MICB diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with Starting Block blocking buffer, and incubated with 50 µl per well of a mouse Human MICA monoclonal antibody starting at a concentration of 2 µg/mL (HA723327) and serially diluting it to a concentration of 0.13 ng/mL for 2 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Rabbit IgG secondary antibody at a dilution of 1:10,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 5 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

Background References

1. Steinle A., Li P., Morris D.L., Groh V., Lanier L.L., Strong R.K., Spies T. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics 53:279-287 (2001)
2. Groh V., Rhinehart R., Secrist H., Bauer S., Grabstein K.H., Spies T. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proc. Natl. Acad. Sci. U.S.A. 96:6879-6884 (1999)