MONOCLONAL ANTIBODY TO
HUMAN NEUTROPHIL DEFENSIN 1-3 (HNP 1-3)
clone D21

Catalog no
HM2058 (lot number and expiry date are indicated on the label)

Description
Human neutrophil defensins (alpha-defensins) belong to the family of cationic trisulfide-containing microbicidal peptides. Besides microbicidal, the peptides exert chemotactic, immunomodulating and cytotoxic activity and participate in host defense and inflammation. Defensins are important effector molecules against enveloped viruses, bacteria, fungi and protozoa, and protein concentrations ranging from 0.5 to 5 µM were shown to kill a wide range of microbes in vitro. Defensins have the ability to attack susceptible microorganisms and destroy the structure of target cell membranes and. Several members of each defensin family were shown to act as microbicides against distinct Gram-negative and Gram-positive bacteria, as well as fungi and viruses in vitro.

Azurophilic granules of neutrophils contain Human Neutrophil Peptide (HNP) 1-4, which are highly homologous. The three principal human defensins, HNP 1-3, are unique to neutrophils and account for about 99% of the total defensin content of these cells. Defensins HNP 1-3 are almost exclusively expressed in neutrophils, therefore it is considered a neutrophil cell marker. Measured amount of defensins is 3-5 microgram per million human neutrophils. When treated with HNP the outer membrane of Escherichia coli becomes permeable. This permeabilization furthermore coincided with the cessation of RNA, DNA and protein synthesis, and with a decreased bacterial viability. Defensins are relatively resistant to proteolysis, low pH and boiling. Activation of neutrophils leads to rapid release of HNP. HNP can be measured in plasma and other body fluids during infection and inflammation. In normal plasma very low levels of HNP are present. Activation of polymorphonuclear leukocytes (PMN) in plasma, as occurs during clotting of blood, leads to a rapid release of HNP.

Anti HNP 1-3 antibody clone D21 recognizes natural HNP 1-3 in biological solutions by means of ELISA in tissue sections and leukocyte smears fixed with ethanol, methanol/acetone or paraformaldehyde, in flow cytometry analysis of human neutrophils stained by cell permeabilization method and in Western-blotting (non-reduced). Furthermore the antibody is cross reactive with Rhesus monkey and cynomolgous macaques HNP1-3.

Aliases
α-defensins, HNP1-3

Species
Mouse IgG1

Cross reactivity
Cross reactant Reactivity
Monkey (Rhesus monkey and cynomolgous macaques) Yes

Formulation
1 ml (100 µg/ml) 0.2 µm filtered antibody solution in PBS, containing 0.1% bovine serum albumin and 0.02% sodium azide.

Application

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N.D. = Not Determined; F = Frozen sections; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IF = Immuno Fluorescence; IP = Immuno Precipitation; P = Paraffin sections; W = Western blot

Application notes
F: FCS blocked sections were incubated with D21 antibody 1:500
FC: Cells were fixed and permeabilized, incubation with primary antibody for 30 min.
FS: In vitro. 0.5ug/ml, 30min -24h at 37°C
IF: 1% formaldehyde fixed cells were stained with 5µl Ab/million cells for 15 min.
P: Formalin fixed sections were deparaffinized and blocked with 1% hydrogen peroxide and serum or 5% BSA
W: Blots blocked with 10% serum for 30'. Primary antibody 2h RT 1:100 in TBS/0.01% tween-20
References

5. Zaharatos, G et al; α-Defensins released into stimulated CD8+ T-cell supernatants are likely Derived from residual granulocytes within the irradiated allogeneic peripheral blood mononuclear cells used as feeder. J Acquir Immune Defic Syndr 2004, 35: 993

Use
For immunohistochemistry, flow cytometry and Western blotting, dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

Positive control
Neutrophils

Negative control
HELA cells

Storage and stability
Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year. The exact expiry date is indicated on the label.

Precautions
For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

Also available
HM2059 Biotinylated monoclonal antibody against Human HNP1-3, clone D21