

**CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET**

<b>Product name</b>	HNP1-3, Human, clone D21, biotinylated		
<b>Catalog number</b>	HM2058BT-50UG		
<b>Lot number</b>	-	<b>Expiry date</b>	-
<b>Volume</b>	500 µl	<b>Amount</b>	50 µg
<b>Formulation</b>	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3	<b>Concentration</b>	100 µg/ml
<b>Host Species</b>	Mouse IgG1	<b>Conjugate</b>	Biotin
<b>Endotoxin</b>	N.A.	<b>Purification</b>	Protein G
<b>Storage</b>	4°C		

**Application notes**

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	3	6,8,10	2	2,3,5,9	4	3,6,9	2	7
Yes	•	•	•	•	•	•	•	•
No								
N.D.								

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

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.

- 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
- Positive control: Neutrophils; Negative control: HELA cells.

**General Information**

**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, human Neutrophil Defensin 1-3

**Cross reactivity** Monkey (Rhesus monkey and cynomolgous macaques): Yes.

- References**
1. Lehrer, R et al; Defensins : antimicrobial and cytotoxic peptides of mammalian cells. Ann Rev Immunol 1993, 11 : 105
  2. Zhang, L et al; Contribution of human alpha-Defensin-1, -2 and-3 to the anti-HIV-1 activity of CD8 antiviral factor. Science 2002, 298: 995

3. Trabattoni, D et al; Human  $\alpha$  defensin in HIV-exposed but uninfected individuals. J Acquir Immune Defic Syndr 2004, 35: 455
4. Chang, T et al; CAF-mediated human immunodeficiency virus type 1 transcriptional inhibition is distinct from  $\alpha$ -Defensin-1 HIV inhibition. J Vir 2003, 77: 6777
5. Zaharatos, G et al;  $\alpha$ -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
6. Tao, R et al; Salivary antimicrobial peptide expression and dental caries experience in children. Antimicrob Agents Chemother 2005, 49: 3883
7. Gow, J et al; Agene signature for post-infectious chronic fatigue syndrome. BMC medical genomics 2009, 2:38
8. Matthijsen, R et al; Enterocyte shedding and epithelial lining repair following ischemia of the human small intestine attenuate inflammation. Plos One, 2009, 4: 9
9. Rodriguez-Garcia, M et al; Increased  $\alpha$ -defensins 1-3 production by dendritic cells in HIV-infected individuals is associated with slower disease progression. Plos One 2010, 2: e9436
10. Ishihata, K et al; Expression of antimicrobial peptides and E-cadherin in periapical lesions. Oral Science International 2013, 10: 70

**Storage&stability** Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

**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.

We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

Approved by Manager of QC  
Brenda Teunissen

Date  
04/11/2019

Do you have any questions or comments regarding this product? Please contact us via [support@hycultbiotech.com](mailto:support@hycultbiotech.com).