CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	CpG-C DNA, Human/Mouse		
Catalog number	HC4041		
Lot number	-	**Expiry date**	-
Volume	Reconstitute with distilled/de ionized water	**Activity**	N.A.
Formulation	Lyophilized purified 22-mer CpG ODN	**Amount**	200 nmol (1395 µg)
Host Species	22-mer CpG ODN	**Concentration**	N.A.
Endotoxin	<24 EU/mg	**Purification**	N.A.
Storage	4°C	**Purity**	>95%

Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #								
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

- FS: CpG-C DNA can be used in biological assays in vitro to activate cells. Furthermore, CpG-C DNA can be used as an immune modulating agent. For in vitro stimulation, 0.05 to 3 µM can be used. It is recommended that users test the reagent and determine their own optimal concentrations.

General Information

Description

ODN 2395 is a prototype of the class of CpG-C oligodeoxynucleotides (ODN) and is particularly effective at activating B- and NK cells and inducing IFN-alpha production in vitro of human peripheral blood mononuclear cells (PBMC). The vertebrate immune system has evolved innate immune defense pattern recognition receptors (PRRs) that detect unmethylated cytosine-phosphate-guanine (CpG) motifs within bacterial DNA. Cellular activation by CpG motifs occurs via the Toll signal pathway. The Toll-like receptor-9 (TLR9, CD289) appears to be a major component of the CpG-DNA receptor, acting by direct binding to CpG-DNA, which triggers the induction of cell signaling pathways including the mitogen activated protein kinase (MAPKs) and NFκB, leading to stimulation of various cells of the immune system. The human TLR9 is expressed in B cells and plasmacytoid dendritic cells (PDC). Mice also express TLR9 in the myeloid compartment. Optimal sequences for activating TLR9 vary among species. Synthetic ODN contain CpG-DNA motifs mimicking the immunostimulatory effects of bacterial DNA and can, therefore, be used as immunoprotective agents, vaccine adjuvants and anti-allergic agents. CpG ODN also affects immune tolerance and autoimmunity. Different classes of CpG ODN are characterized each with distinct effects on the immune response: CpG-A ('D'-type), CpG-B ('K'-type), and CpG-C. CpG-C ODN combines the immune effects of CpG-A and -B ODN since they induce strong B-cell activation comparable to CpG-B together with IFN-alpha secretion as CpG-A ODN. Furthermore NK cells isolated from peripheral blood and cultured with CpG-C ODN, in the presence of either IL-12 or IL-8, express higher levels of CD69 as compared to those stimulated with ether CpG-A or CpG-B ODN. Moreover, NK cells cultured with CpG-C ODN displayed higher cytolytic activity against tumor cell lines. In contrast to CpG-A, CpG-C ODN has a complete phosphorothioate (PS) backbone without poly-G motifs, but contains like CpG-A palindromic sequences combined with stimulatory CpG motifs. In vivo studies demonstrate that CpG-C are very potent Th1 adjuvants. Since CpG-C ODN combine the immune effects of both CpG-A and CpG-B, CpG-C might have broader immune therapeutic effects in different tumor types and infectious diseases in which a combination of A- and B-class ODN would be desirable. The prototype sequence of a CpG-C is the 22-mer ODN 2395 that is able to modulate the immune response in both human and mice. It has the following sequence: 5'-t**cg**t**cg**ttt**cg**g**cgcgcg**c**cg**-3'. Regular letters represent PS linkage and bold letters represent CpG dinucleotides.

References

1. Krieg, A; CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol 2002, 20: 709
2. Vollmer, J et al; Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur J Immunol 2004, 34: 251
3. Jurk, M et al; C-class CpG ODN: sequence requirements and characterization of immunostimulatory activities on mRNA level. Immunobiology 2004, 209: 141
4. Sivori, S et al; Comparison of different CpG oligodeoxynucleotide classes for their capability to stimulate human NK cells. Eur J Immunol 2006, 36: 1-7

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Storage&stability	Caution: vial is under vacuum. Lyophilized product should be stored at 4°C. Store stock solution in aliquots at –20°C. Repeated freeze and thaw cycles will cause loss of activity. Under recommended storage conditions, product is stable for 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	Date
Robbert Zwinkels	29/03/2018

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