# CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

| | | | |
|---|---|---|---|
| **Product name** | CpG-B DNA, Human/Mouse | | |
| **Catalog number** | HC4039 | | |
| **Lot number** | - | **Expiry date** | - |
| **Volume** | Reconstitute with distilled/de ionized water | **Activity** | N.A. |
| **Formulation** | Lyophilized purified 24-mer CpG ODN | **Amount** | 200 nmol (1524 µg) |
| **Host Species** | 24-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 # | | | | | 2-10 | | | |
| 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-B DNA can be used in biological assays in vitro to activate cells. Furthermore, CpG-B 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 2006 is a prototype of the class of CpG-B oligodeoxynucleotides (ODN), also known as 'K'-type ODN, with a full phosphorothioate (PS) backbone. It is particularly effective for activating B cells. 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-B ODN are characterized by a full phosphorothioate backbone with one or more CpG motifs without poly –G motifs. CpG-B ODN are weak inducers of IFN-alpha but are very potent Th1 adjuvants and strong B cell response stimulators. CpG-B ODN promote survival, activation, and maturation of both monocyte derived dendritic cells and PDC. The prototype sequence of CpG-B is the 24-mer ODN 2006 that is able to modulate the immune response in both human and mice. It has the following sequence: 5'-tcgtcgttttgtcgttttgtcgtt-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. Wang, J et al; CpG-independent synergistic induction of beta-chemokines and a dendritic cell phenotype by orthophosphorothioate ODN and GM-CSF in elutriated human primary monocytes. J Immunol 2005, *174*: 6113
4. Heidt, S et al; Intravenous immunoglobulin preparations have no direct effect on B cell proliferation and immunoglobulin production. Clin Exp Immunol 2009, *158*:99
5. Capolunghi, F et al; Pharmacological inhibition of TLR9 activation blocks autoantibody production in human B cells from SLE patients. Rheumatology 2010, *49*:2281
6. Saxena, M et al; CpG Protects Human Monocytic Cells against HIV-Vpr–Induced Apoptosis by Cellular Inhibitor of Apoptosis-2 through the Calcium-Activated JNK Pathway in a TLR9-Independent Manner. J Immunol 2011, *187*:5865
7. Tadema, H et al; Bacterial DNA motifs trigger ANCA production in ANCA-associated vasculitis in remission. Rheumatology 2011, *50*:689
8. Tadema, H et al; Increased Expression of Toll-Like Receptors by Monocytes and Natural Killer Cells in ANCA-Associated Vasculitis, PLoSOne 2011, *6*: e24315

9. Rahim, M et al; Ly49Q Positively Regulates Type I IFN Production by Plasmacytoid Dendritic Cells in an Immunoreceptor Tyrosine–Based Inhibitory Motif–Dependent Manner. J Immunol 2013, *190*:3994
10. Lepse, N et al; Altered B cell balance, but unaffected B cell capacity to limit monocyte activation in anti-neutrophil cytoplasmic antibody-associated vasculitis in remission. Rheumatology 2014, *53*:1683

**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 |

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.