

InvivoGen Insight

Inside this issue:

Products

Vaccine Adjuvants

- ▶ Stimulatory CpG ODNs
- ▶ DNA vaccine booster

TLR Inhibition

- ▶ Suppressive ODNs
- ▶ Inhibitors of TLR Signaling

Antibody Generation

- ▶ pFUSE-CLg & pFUSE-CHlg

Cellular Promoters

- ▶ PromTest™



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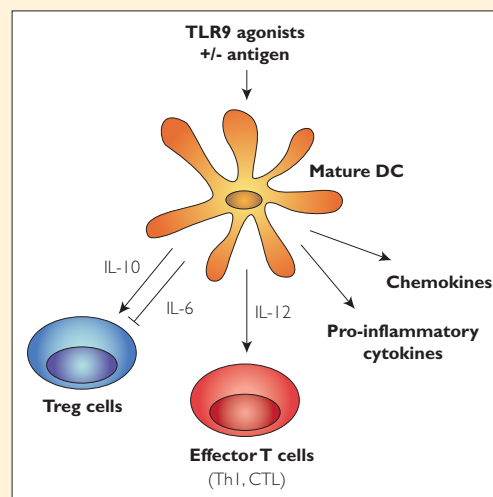
TLR9 agonists: double-edge sword for immune therapies

Toll-like receptor 9 (TLR9) senses unmethylated CpG dinucleotides, a hallmark of microbial DNA, that can be mimicked by synthetic oligonucleotides containing CpG motifs (CpG ODNs). TLR9 stimulation by CpG DNA or CpG ODNs triggers intracellular signaling leading to the activation of macrophages, dendritic cells (DC) and B cells, and the production of cytokines, chemokines, and immunoglobulins. Subsequently, cytokines produced by DC, such as IL-12, induce the differentiation of naive T cells into T helper 1 (Th1) and cytotoxic T-cells (CTL). Therefore, TLR9 agonists can elicit innate immune defenses and antigen T-cell specific responses, a property that underlines their development as vaccine adjuvants or immunotherapeutics for infectious diseases and cancer.

Studies in animal models have demonstrated that the immune defenses mounted by CpG ODNs alone or as vaccine adjuvants can protect against a variety of viral, bacterial, and parasitic diseases¹. Promising results in the prophylactic treatment of hepatitis B have been obtained from phase III trials with a combination of a CpG ODN and hepatitis B surface antigen (HepB) antigen².

Antitumor activity of CpG ODNs has also been established in numerous mouse models. Encouraging results in the treatment of cancers have come from phase I and II clinical trials using CpG ODNs as a tumor vaccine adjuvant, monotherapy, or in combination with chemotherapy². However, there have been also some disappointing results with one pharmaceutical company recently dropping its clinical program with a TLR9 agonist in non-small cell lung cancer. The interim data of two phase 3 trials of PF-3512676 (formerly called CpG 2006, see page 2) showed that it failed to improve the clinical outcomes compared to chemotherapy alone³.

A major obstacle to the development of effective immunotherapeutics to cancer is the immunosuppressive environment of the tumor. Indeed, tumors secrete a range of molecules that inhibit effector immune responses but also induce immune cells that have suppressive activity such as regulatory T (Treg) cells. TLR agonists, including CpG ODNs, have been shown to break tolerance to self-antigens by inhibiting the function of Treg cells via the production of IL-6 by DC⁴. However, CpG ODNs have also been shown to stimulate the production of IL-10 by DC, and promote the induction of Treg cells. Therefore, CpG ODNs can generate effector as well as suppressive immune responses. Jarnicky *et al.* have demonstrated that the immunosuppressive arm of CpG ODN-induced TLR activation can be selectively blocked by inhibition of p38 MAPK, an intermediate in the IL-10 production by DC⁵. Incubation of CpG-ODN-activated Ag-pulsed DC with SB203580 (see page 2) suppressed their ability to generate Treg cells while enhancing the induction of Th1 cells.



TLR9-mediated effector and suppressive immune responses

Recent reports indicate that TLR9 may play a role in the pathogenesis of various autoimmune diseases, such as systemic lupus erythematosus (SLE). Under certain conditions, TLR9 is able to recognize self-DNA leading to the production of anti-DNA autoantibodies. This discovery has prompted the development of specific inhibitors of TLR9. Paralleling the approach of stimulating TLR9 with CpG ODNs, it was found that suppressive ODNs exist that are able to inhibit TLR9 activation. The most potent inhibitory sequences contain TTAGGG multimers found in mammalian telomeres or a 5' CCT, a C-free linker four to five bases long, and a GGG(G) tail (see page 2). Some of these suppressive ODNs are able to inhibit an already ongoing immune response and therefore could be useful in the treatment of SLE⁶.

This data illustrate the great potential of TLR9-based drugs for the treatment of infectious diseases, cancer and autoimmune diseases. However, as they can activate both the effector and suppressive arms of the immune system, more studies are needed to better understand the mechanisms involved allowing the development of safer and more effective TLR therapeutics.

1. Krieg AM., 2007. Antiinfective applications of toll-like receptor 9 agonists. *Proc Am Thorac Soc.* 4(3):289-94. 2. Schmidt C., 2007. Clinical setbacks for toll-like receptor 9 agonists in cancer. *Nat Biotechnol.* 25(8):825-6. 3. Pasare C. & Medzhitov R., 2003. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science.* 299(5609):1033-6. 4. Jarnicky AG. *et al.*, 2008. Attenuating regulatory T cell induction by TLR agonists through inhibition of p38 MAPK signaling in dendritic cells enhances their efficacy as vaccine adjuvants and cancer immunotherapeutics. *J Immunol.* 180(6):3797-806. 5. Peter M. *et al.*, 2008. Characterization of suppressive oligodeoxynucleotides that inhibit Toll-like receptor-9-mediated activation of innate immunity. *Immunology.* 123(1):118-28.

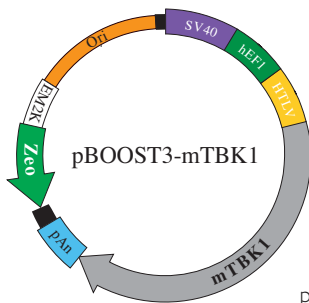
Vaccine Adjuvants

Stimulatory CpG ODNs

CpG ODNs activate TLR9 inducing both innate immune defenses and antigen-specific T-cell responses that can be harnessed for improving vaccines. InvivoGen provides a selection of CpG ODNs, guaranteed endotoxin-free and tested for activity in various TLR9-expressing cell lines. These CpG ODNs are available in different sizes.

Product	Description	Qty	Cat. Code
ODN 1585	Stimulatory CpG-ODN type A Mouse specific	200 µg 1 mg 5 mg	tlr1-modna tlr1-modna-1 tlr1-modna-5
ODN 1668	Stimulatory CpG-ODN type B Mouse specific	200 µg 1 mg 5 mg	tlr1-modnb tlr1-modnb-1 tlr1-modnb-5
ODN 1826	Stimulatory CpG-ODN type B Mouse specific	200 µg 1 mg 5 mg	tlr1-modn tlr1-modn-1 tlr1-modn-5
ODN 2006	Stimulatory CpG-ODN type B Human specific	200 µg 1 mg 5 mg	tlr1-hodnb tlr1-hodnb-1 tlr1-hodnb-5

Product	Description	Qty	Cat. Code
ODN 2216	Stimulatory CpG-ODN type A Human specific	200 µg 1 mg 5 mg	tlr1-hodna tlr1-hodna-1 tlr1-hodna-5
ODN 2336	Stimulatory CpG-ODN type A Human specific	200 µg 1 mg 5 mg	tlr1-hodna2 tlr1-hodna2-1 tlr1-hodna2-5
ODN 2395	Stimulatory CpG-ODN type C Human/mouse	200 µg 1 mg 5 mg	tlr1-odnc tlr1-odnc-1 tlr1-odnc-5
ODN M362	Stimulatory CpG-ODN type C Human/mouse	200 µg 1 mg 5 mg	tlr1-hodnc tlr1-hodnc-1 tlr1-hodnc-5



DNA Vaccine Booster **NEW**

TANK-binding kinase 1 (TBK1), a non-canonical I κ B kinase, was recently shown to mediate the adjuvant effect of DNA vaccines. Administration of DNA vaccines induces the production of type I interferons and inflammatory cytokines in a CpG-independent manner but in a TBK1-dependent manner. Therefore, co-administration of a TBK1-expressing plasmid is expected to further boost DNA vaccine-induced immunogenicity.

Ishii KJ. *et al.*, 2008. TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. *Nature* 451: 725-729

pBOOST3-mTBK1 plasmid expresses the mouse TBK1 gene. Expression of the transgene is driven by a strong composite promoter composed of the SV40 enhancer, EF-1 α core promoter and HTLV 5'UTR. The plasmid is selectable with Zeocin™ in *E. coli*. pBOOST3-mTBK1 plasmid is provided as 20 µg of lyophilized DNA. **Catalog code #pbst3-mtbk1**

TLR Inhibition

Suppressive ODNs

DNA sequences that inhibit the activation of TLR9 by stimulatory CpG ODNs have been identified. The most potent inhibitory sequences are (TTAGGG) $_4$ found in mammalian telomeres and ODN 2088 which derives from a murine stimulatory CpG ODN by replacement of 3 bases. Recently, another suppressive, guanosine-rich ODN, named G-ODN, was described. G-ODN was suppressive in murine DC and macrophages as well as in human plasmacytoid DC.

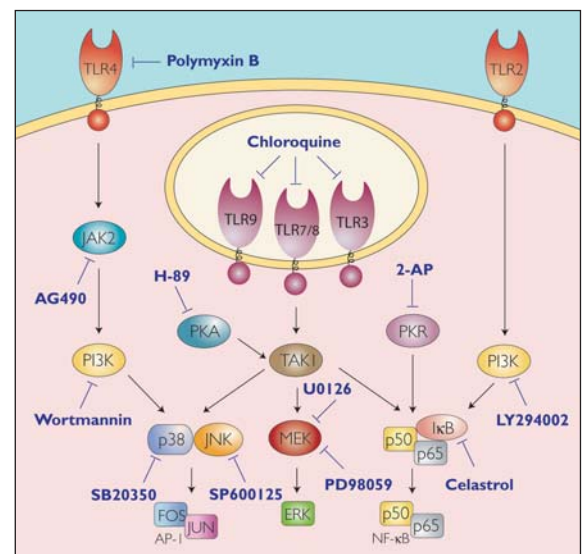
Product	Sequence	Qty	Cat. Code
ODN 2088	5'-tcctggcggggaagt-3'	200 µg	tlr1-minhodn
ODN TTAGGG	5'-tttagggttagggttaggg-3'	200 µg	tlr1-hinhodn
G-ODN NEW	5'-ctcctattgggggttcctat-3'	200 µg	tlr1-godn

Peter M. *et al.*, 2007. Characterization of suppressive oligodeoxynucleotides that inhibit Toll-like receptor-9-mediated activation of innate immunity. *Immunology* 123(1):118-28.

TLR Signaling Inhibitors

Inhibitors of TLR signaling are molecules that intervene in the different steps of the TLR activation and signaling cascade, including TLR binding, TLR relocalization and MAP kinases and transcription factors activation.

Product	Description	Qty	Cat. Code
AG490 NEW	JAK2 inhibitor	10 mg	tlr1-ag4
2-Aminopurine	PKR inhibitor	250 mg	tlr1-apr
Celastrol NEW	I κ B inhibitor	1 mg	ant-clc
Chloroquine	Endosomal acidification inhibitor	250 mg	tlr1-chq
H-89 NEW	PKA inhibitor	5 mg	tlr1-h89
LY294002	PI3K inhibitor	5 mg	tlr1-ly29
PD98059	MAP kinase kinase inhibitor	10 mg	tlr1-pd98
Polymyxin B	LPS-induced TLR4 activation inhibitor	100 mg	tlr1-pmb
SB203580	p38/RK MAP kinase inhibitor	5 mg	tlr1-sb20
SP600125 NEW	JNK inhibitor	10 mg	tlr1-sp60
U0126	MEK1-MEK2 inhibitor	5 mg	tlr1-u0126
Wortmannin NEW	PI3K inhibitor	5 mg	tlr1-wtm



Antibody Generation NEW

pFUSE-CLIg and pFUSE-CHlg

- Isotype switch to generate IgG antibodies with different effector functions
- Generation of entire IgG antibodies, chimeric, humanized or fully human

pFUSE-CLIg and pFUSE-CHlg plasmids are designed to change a monoclonal antibody from one isotype to another human or murine IgG isotype therefore enabling the generation of antibodies with the same antigen affinity but with different effector functions (increased or reduced ADCC and CDC). Furthermore, they can be used to produce entire IgG antibodies from Fab or scFv fragments that are either chimeric, humanized or fully human depending on the nature of the variable region.

Principle

Antibodies are dimeric proteins composed of two light and heavy chains, each comprising a constant region and a variable region. pFUSE-CHlg and pFUSE-CLIg express the constant regions of the heavy (CH) and light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody. Transfection of mammalian cell lines with the recombinant pFUSE-CHlg and pFUSE-CLIg pair allows to generate an IgG antibody that can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

VH and VL sequences

To obtain the cDNA sequence of the VH and VL regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the "known" CH and CL regions. Alternatively 5' RACE can be used. The resulting amplicons are sequenced.

Cloning into pFUSE-CHlg and pFUSE-CLIg

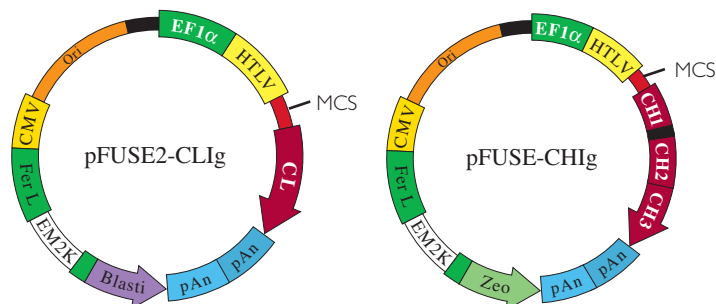
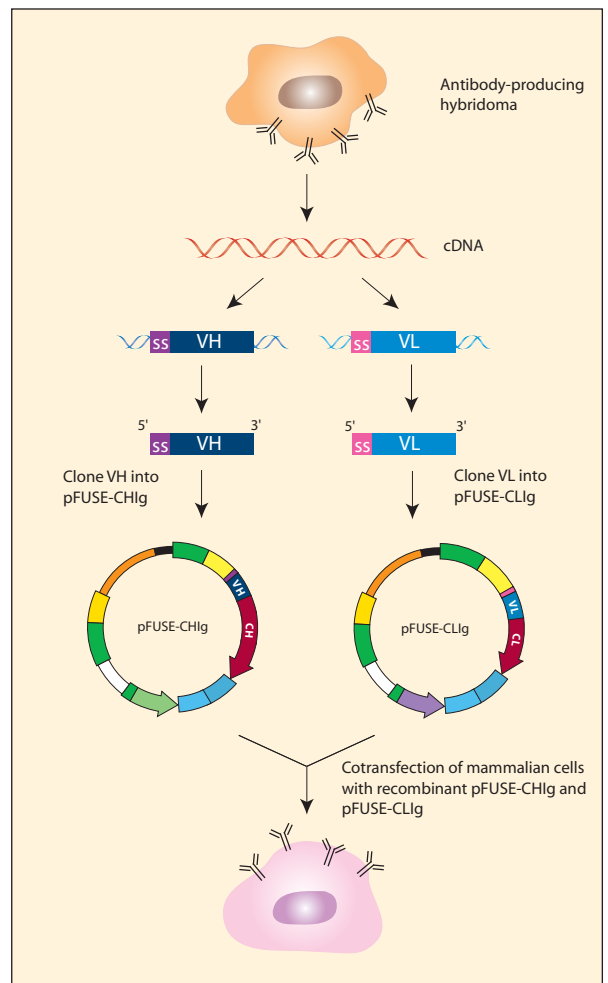
Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. When generating the insert for VH, a Nhe I site must be introduced at the 3' end to maintain the integrity of the constant region. Similarly, when generating the insert for VL, a Bsi WI (human VL) or Bst API (mouse VL) site must be introduced at the 3' end. At the 5' end, there is a choice of restriction sites: Eco RI, Eco RV, or Xho I for VH, and Age I, Bst EI, or Nco I for VL.

Plasmid Features

- pFUSE2-CLIg plasmids feature the constant region of the human or mouse kappa light chain. They are selectable with blasticidin.
- pFUSE-CHlg plasmids feature the heavy chain constant region of the human or mouse IgG isotypes.
 - Human isotypes: IgG1, IgG2, IgG3 and IgG4
 - Murine isotypes: IgG1, IgG2A, IgG2B and IgG3
 pFUSE-CHlg plasmids are selectable with Zeocin™.

pFUSE-CLIg and pFUSE-CHlg plasmids are provided as 20 µg of lyophilized DNA.

Antibody generation using pFUSE-CHlg and pFUSE-CLIg



Product	Isotype	Qty	Cat. Code
pFUSE2-CLIg-hk	Human kappa	20 µg	pfuse2-hclk
pFUSE2-CLIg-mk	Mouse kappa	20 µg	pfuse2-mclk
pFUSE-CHlg-hG1	Human IgG1	20 µg	pfuse-hchg1
pFUSE-CHlg-hG2	Human IgG2	20 µg	pfuse-hchg2
pFUSE-CHlg-hG3	Human IgG3	20 µg	pfuse-hchg3
pFUSE-CHlg-hG4	Human IgG4	20 µg	pfuse-hchg4
pFUSE-CHlg-mG1	Mouse IgG1	20 µg	pfuse-mchg1
pFUSE-CHlg-mG2a	Mouse IgG2a	20 µg	pfuse-mchg2a
pFUSE-CHlg-mG2b	Mouse IgG2b	20 µg	pfuse-mchg2b
pFUSE-CHlg-mG3	Mouse IgG3	20 µg	pfuse-mchg3

PromTest™ **NEW**

Quickly find the best promoter for your cell line

- Ten ready-to-use promoters with different levels of expression
- Drive GFP expression for convenient monitoring of promoter strength
- Cost-effective

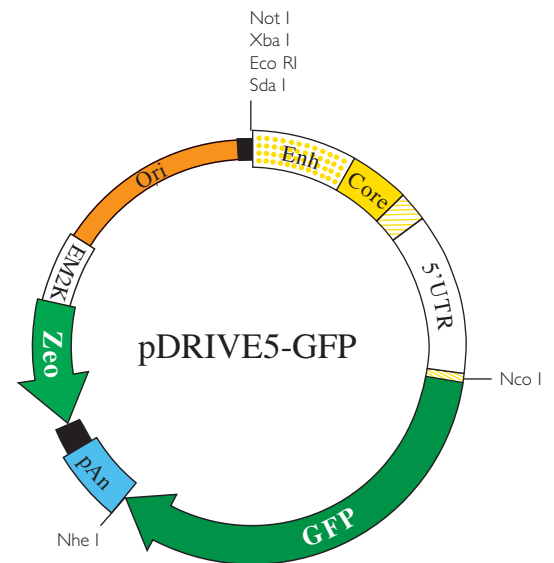
PromTest™ is a collection of ten ubiquitous composite promoters provided in the pDRIVE5-GFP plasmid. These composite promoters were generated by assembling enhancers, core promoters and 5'UTRs of different origins. The activity of each combination depends on the cellular context (see graphs).

pDRIVE5-GFP features a GFP reporter gene for convenient monitoring of promoter activity. The strength of each promoter can be assessed qualitatively by fluorescence microscopy and quantitatively using a fluorometer or flow cytometry.

pDRIVE5-GFP plasmids are selectable in *E. coli* with Zeocin™.

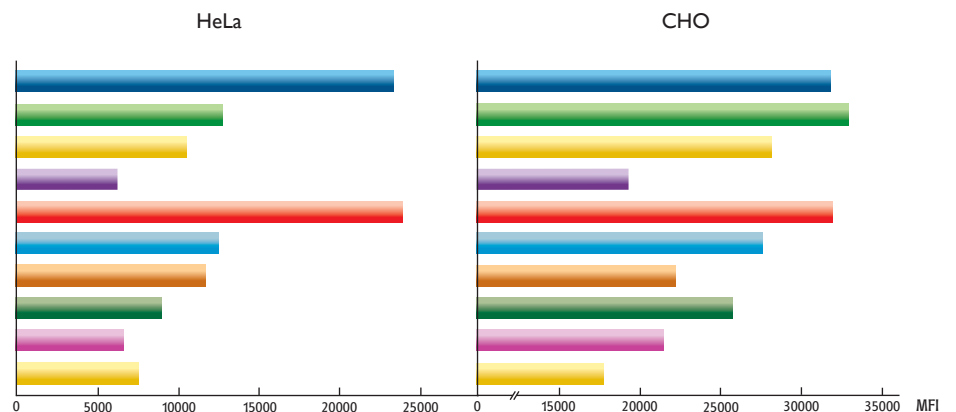
PromTest™ contains 5 µg of each plasmid, enough for multiple transfections using your favorite reagent or technique. pDRIVE5-GFP plasmids can be amplified in any common *E. coli* laboratory strains. They are also available individually as 20 µg high-quality endofree DNA.

Once you have determined the best promoter for your cell line and application, you can either replace the GFP gene with your gene of interest, or subclone the promoter into another plasmid with mammalian selection, such as InvivoGen's pSELECT.



Product	Quantity	Cat. Code
PromTest™	10 x 5 µg	prom-test
pDRIVE5-GFP-n	20 µg	pdv5-gfp<n>

Plasmid	Enhancer	Core Promoter	5' UTR
pDRIVE5-GFP-1	hCMV	hEF1	HTLV
pDRIVE5-GFP-2	mCMV	hEF1	HTLV
pDRIVE5-GFP-3	SV40	hEF1	HTLV
pDRIVE5-GFP-4	mTyr	hEF1	HTLV
pDRIVE5-GFP-5	hCMV	hCMV	HTLV
pDRIVE5-GFP-6	SV40	hCMV	HTLV
pDRIVE5-GFP-7	hCMV	hFerL	chEF1
pDRIVE5-GFP-8	mCMV	hFerL	chEF1
pDRIVE5-GFP-9	SV40	hFerL	chEF1
pDRIVE5-GFP-10	hAldA	hFerL	chEF1



Evaluation of PromTest™ in different cell lines: HeLa (human cervical cancer) and CHO (chinese hamster ovary) cells were transiently transfected with each of the 10 pDRIVE5-GFP plasmids of PromTest™ using LyoVec™ (cat. code: lyec-1). The strength of the various promoters was analyzed by flow-cytometry 48h after transfection.

Abbreviations: chEF1, chimpanzee elongation factor 1 alpha; hAldA, human aldolase A; hCMV, human cytomegalovirus; hEF1, human elongation factor 1 alpha; hFerL, human ferritin light chain; HTLV, human T lymphocyte virus; mCMV, mouse cytomegalovirus; mTyr, mouse tyrosinase; SV40, simian virus 40.