Caithness BIOTECHNOLOGIES

High quality reagents for innate immunity research, vaccine development and drug discovery

Recombinant flagellin fusion proteins

Background

Flagellin is the principal structural protein of bacterial flagella, the helical appendages that enable bacterial motility [1]. Almost uniquely among proteins, flagellin contains regions that are sufficiently conserved across bacterial species to be recognised by two distinct pattern recognition receptors (PRRs) of the mammalian innate immune system. Flagellin may bind Toll-like receptor 5 (TLR5) on the surface of immune cells, triggering signalling pathways that result in the production of pro-inflammatory cytokines and other immune responses [2]. Alternatively, flagellin that enters the cytosol may be recognised by the NAIP / NLRC4 inflammasome, resulting in the processing of pro-IL1β to the active form of IL1β [3]. As these responses make flagellin a potent activator of dendritic cells and adaptive immune responses more generally, it has received much interest as a vaccine adjuvant and carrier in both pre-clinical models and clinical trials [4].

Caithness Biotech offers recombinant flagellin proteins, covalently fused to bespoke antigens of interest, for studies of innate immune signalling, host-pathogen interactions, immunoassays, and as an adjuvant and carrier for vaccine development (Figure 1). Uniquely, our flagellin proteins are expressed in mammalian cells to maximise authenticity of the partner antigen structure and minimise the presence of contaminating bacterial stimulants of other TLRs.

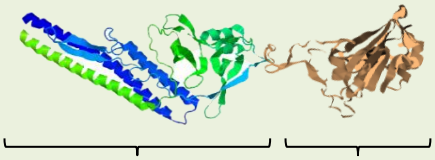


Figure 1: Schematic of typical flagellin fusion protein organisation

Key advantages of our flagellin proteins

- ✓ Platform shown previously to induce diverse humoral (IgM, IgG, IgA), and cell-mediated (T_H1, T_H2 $CD4^+$ helper T-cell and $CD8^+$ cytotoxic T-cell), responses to the covalently linked target antigen
- ✓ Bespoke antigen cloning available
- ✓ Expression in mammalian cells for extremely low levels of non-specific PAMP contaminants
- ✓ Each batch tested to validate capacity to potently stimulate TLR5-signalling
- ✓ Human glycosylation patterns for authentic structure, stability and antigenicity of partner antigens
- ✓ Potential for intranasal delivery and induction of mucosal immunity
- ✓ Combined adjuvant and carrier platform

Advantages of combined adjuvant and carrier

Subunit vaccines benefit from improved potential for safety and ease of manufacture in comparison to live-attenuated vaccines, but often require two additional components for vaccine efficacy - an adjuvant and a carrier. The adjuvant serves to stimulate innate immune receptors to trigger DC activation, maturation and antigen presentation. The carrier protein contains peptide sequences which are readily presented on MHC molecules of DCs to generate the T-cell help necessary to support effective immune responses to antigens, such as polysaccharides, which may not otherwise efficiently induce T-cell help.

Flagellin proteins are capable of serving as both adjuvant and carrier for protein antigens covalently attached to them as fusion partners [4]. This linkage ensures that antigen, adjuvant and carrier all enter the same endosomal compartment at the same time, a process that has been shown to generate far more robust immune responses than antigens simply admixed with carrier or adjuvant [5].

Caithness Biotechnologies Ltd., 72 Boston Road, Leicester, UK, LE4 1HB.
Tel: +44 (116) 326 3802| Company: 9395037 | VAT : 202 4512 64
contact@caithnessbiotechnologies.com | www.caithnessbiotechnologies.com

Recombinant flagellin fusion proteins

A proven adjuvant with low toxicity

Systemic exposure to some TLR-stimulants, such as the TLR4 agonist lipopolysaccharide (LPS), can result in the damaging systemic inflammatory response syndrome (SIRS), caused by the excessive release of pro-inflammatory cytokines from myeloid cells. However, the systemic response to flagellin, even at high doses, does not seem to induce such a response. It is thought this may be because of the profile of TLR5 expression (mainly on epithelial cells and DCs) and lower induction of the key inflammatory cytokines IL1β and TNFα [6]. Flagellin proteins, either in the native form or as recombinant fusion proteins, have also been administered to human volunteers in dozens of small scale trials since the 1960s, with either minor or no side effects reported [7,8]. Thus, the flagellin-fusion platform has a long track record of use with low toxicity.

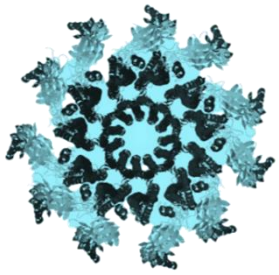


Figure 2: Flagellin monomers have the potential to spontaneously multimerise resulting in the potential for additional stimulation of B-cells via B-cell receptor cross-linking by the fusion partner antigen (FljB filament shown at left).

Capacity to induce diverse immune responses

The administration of flagellin fusion proteins to mice has been shown to induce a mixed antibody response including induction of IgM, IgG1, IgG2a/IgG2c and IgA antibodies specific for the partner antigens [9]. Flagellin activates DCs *in vivo* and contains its own $CD4^+$ T-cell epitopes [10]. It furthermore promotes $CD4^+$ T-cell responses to peptides of its fusion partners [11], stimulating both T_H1 and T_H2 based responses to the partner antigen [10,11]. Flagellin also contains $CD8^+$ T-cell epitopes, and promotes $CD8^+$ T-cell responses to partner antigens when these are covalently attached to the flagellin molecule [5,11,12]. Flagellin fusion proteins have been shown to induce protective immunity in diverse pre-clinical models of viral or bacterial challenge [9].

Diverse potential routes of administration

Flagellin, and its fusion proteins, have been shown to be capable of inducing protective immunity when given subcutaneously, intraperitonealy, intramuscularly and intranasally [13]. The latter option raises the possibility of needle-free vaccination with the additional advantage of inducing mucosal immunity, including an IgA response, which is limited in response to most other subunit vaccines.

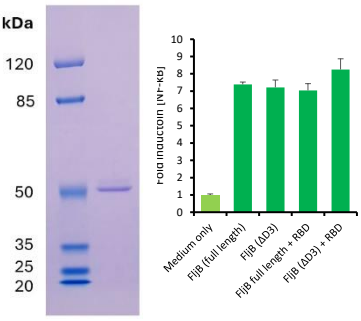
Advantages of our flagellin proteins

Caithness Biotech recombinant flagellins are based on the FljB protein of *Salmonella enterica* subsp. *enterica* serovar Typhimurium. We offer the full-length native protein, the protein lacking the D3 hypervariable domain, and fusion proteins placing your antigen of interest at either the C-terminus, or in place of the D3 domain.

We test every batch using a HEK-293 cell TLR5 reporter assay to ensure potent activation by TLR5 of each product. Uniquely, our proteins are expressed in mammalian cells to maximise purity and minimise presence of contaminating bacterial stimulants of other TLRs, such as TLR2 and TLR4.

Potential applications of our flagellin proteins include use in studies of innate immune signalling, host-pathogen interactions, as an antigen for ELISA and as an adjuvant, carrier and fusion partner for vaccine development.

Figure 3: SDS PAGE analysis of ΔD3 flagellin, and typical results of HEK-293-TLR5 reporter analysis of capacity of FljB variants to stimulate TLR5, in the presence or absence of fusion partner antigen (RBD).



References

[1] Samatey FA, et al. Nature 410:331-7 (2001) [2] Smith KD, et al. Nat Immunol 4:1247-53 (2003) [3] Zhao Y, et al. Nature 477:596-600 (2011) [4] Huleatt JW, et al. Vaccine 25:763-75 (2007) [5] [5380] Bates JT, et al. J Immunol 186:6255-6262 (2011) [6] Burdelya LG, et al. Science 320:226-30 (2008) [7] Rowley MJ, Mackay IR. Clin Exp Immunol 5:407-418 (1969) [8] Taylor DN, et al. Vaccine 29:4897-902 (2011) [9] Hajam IA, et al. Exp Mol Med 49:e373 (2017) [10] McSorley SJ, et al. J Immunol 169:3914-3919 (2002) [11] Cuadros C, et al. Infect Immun 72:2810-2816 (2004) [12] Huleatt JW, et al. Vaccine 25:763-75 (2007) [13] Song L, et al. PLoS One. 3:e2257 (2008)

Caithness Biotechnologies Ltd., 72 Boston Road, Leicester, UK, LE4 1HB.
Tel: +44 (116) 326 3802| Company: 9395037 | VAT : 202 4512 64
contact@caithnessbiotechnologies.com | www.caithnessbiotechnologies.com