Recombinant *Salmonella enterica* serovar Typhimurium FljB flagellin protein (ΔD3)

Description	
Product	Recombinant *Salmonella enterica* serovar Typhimurium FljB protein (ΔD3)
Catalogue number	FLA-01
Size / volume	20 µg
Expression system	HEK-293 cells
Amino acids	Met 1 to Ala 190, GS linker, Val 293 - Arg 506, accession number P52616
Tags	C-terminal 6x His tag
Sequence graphic	
Intended use	For laboratory research only, not for clinical or diagnostic use.

Specifications	
Format	Lyophilised from sterile PBS (pH 7.4) with trehalose as protectant and without additional carrier protein.
Purity	>95% by SDS PAGE
Molecular weight	Migrates at ~ 51 kDa (glycosylation present)
LPS content	< 0.1 ng / µg (by HEK-293-TLR4 bioassay, relative to *E. coli* LPS standard)
BLP content	< 0.1 ng / µg (by HEK-293-TLR2 bioassay, relative to Pam_3CSK_4 standard)
Amino acid sequence	AMAQVINTNSLSLLTQNNLQKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKGLTQASRNANDGISIAQTT EGALNEINNNLQRVRELAVQSAQSTNSQSDLDSIQAEITQRLNEIDRVSGQTQFNGVKVLAQDNTLTIQVGANDGETID IDLKQINSQTLGLDSLNVQKAYDVKDTAVTTKAGGGGSTSASGGGSVVSADAKNALIAGGVDATDANGAELVKMSYTDK NGKTIEGGYALKAGDKYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKTYQASKAAGHDFKA QPELAEAAAKTTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNQLSEARSRIEDSDYATEVSQMSRAQIL QQAGTSVLAQANQVPQNVLSLLRGSHHHHHH
Applications	ELISA / bioassay / SDS PAGE / binding studies / immunoassays

Reconstitution and storage	
Stability	The product is stable in lyophilised format for several weeks at room temperature, although we recommend storage at -20°C prior to reconstitution.
Reconstitution	Centrifuge vial briefly to allow contents to settle. Reconstitute in 40 µl sterile PBS and resuspend by pipetting up and down gently several times to yield a protein concentration of 500 µg/ml. Allow to fully solubilise for 5 minutes at RT before use.
Storage	Aliquot and store at 4°C for up to 1 week, -20°C for up to 1 month or at -80°C for up to 12 months. Avoid repeated freeze thaw cycles which may impact on protein activity.

Data

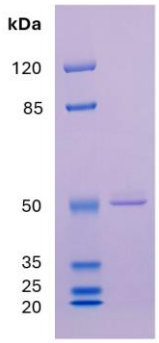

Figure 1: SDS PAGE analysis
1 µg of recombinant protein was separated by reducing SDS PAGE and visualised by Coomassie Blue staining. Caithness Biotech recombinant FljB ΔD3 migrates at approximately 51 kDa due to glycosylation.

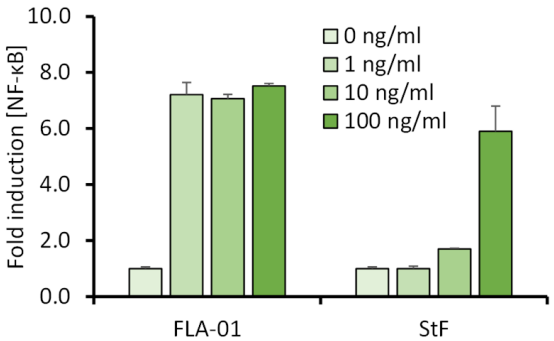

Figure 2: Validation of the capacity of recombinant FljB to stimulate TLR5-signalling
HEK-293 cells were transfected with NF-κB reporter and TLR5, then treated with indicated concentrations of the reconstituted protein (FLA-01) or native (non-recombinant) *S. typmimurium* flagellin (StF) overnight. NF-κB activation was measured by luminometry.

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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, and pyroptosis via cleavage of gasdermin D [3]. As these responses make flagellin a potent activator of dendritic cells and adaptive immunre responses more generally, it has received much interest as a vaccine adjuvant and carrier in both pre-clinical models and clinical trials [4]. The flagellin molecule can be thought of as comprising four major domains, with domains D0 and D1 being highly conserved, and containing the motifs responsible for recognition by TLR5 and NLRC4. The D3 domain, by contrast, is highly variable, and is the dominant epitope for anti-flagellin antibodies arising from natural infection as it is exposed on the surface of the flagellar filament. Fusion proteins in which antigens of interest either replace the D3 domain, or are attached at the C-terminus of flagellin, have been shown to be potent inducers of humoral and T-cell responses to the target antigen [4].

Caithness Biotech recombinant *Salmonella enterica* serovar Typhimurium ΔD3 flagellin comprises amino acids Met 1 to to Ala 190, a short glycine-serine linker, then Val 293 to Arg 506 of *S.* Typhimurium FljB protein. The product comprises all domains of the native protein except for the hypervariable D3 domain (ΔD3). It is a potent stimulus of TLR5, and 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 ΔD3 flagellin protein include use in studies of innate immune signalling, host-pathogen interactions, as an antigen for ELISA and as an adjuvant, carrier or fusion partner for vaccine development.

References

1)	Samatey FA, Imada K, Nagashima S, Vonderviszt F, Kumasaka T, Yamamoto M, Namba K. Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. Nature 410:331-7 (2001)
2)	Smith KD, Andersen-Nissen E, Hayashi F, Strobe K, Bergman MA, Barrett SLR, Cookson BT, Aderem A. Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat Immunol 4:1247-53 (2003)
3)	Zhao Y, Yang J, Shi J, Gong Y-N, Lu Q, Xu H, Liu L, Shao F. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature 477:596-600 (2011)
4)	Huleatt JW, Jacobs AR, Tang J, Desai P, Kopp EB, Huang Y, Song L, Nakaar V, Powell TJ. Vaccination with recombinant fusion proteins incorporating Toll-like receptor ligands induces rapid cellular and humoral immunity. Vaccine 25:763-75 (2007)

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