Recombinant *S.* Typhimurium FljB protein with C-terminal SARS-CoV2 RBD domain

Description	
Product	Recombinant *Salmonella enterica* serovar Typhimurium FljB flagellin protein with C-terminal SARS-CoV2 RBD domain
Catalogue number	FLB-R1
Size / volume	20 µg
Expression system	HEK-293 cells
Amino acids	*Salmonella* FljB Met 1 to Arg 506, SARS-CoV2 Arg 319 to Phe 541, accession numbers P52616 and NCBI reference sequence YP_009724390.1
Tags	C-terminal 6x His tag
Sequence graphic	*S. typhimurium FljB* flagellin aa 1 -506 | SARS-CoV2 RBD aa 319 - 541 | 6x His
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 ~ 84 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 IDLKQINSQTLGLDSLNVQKAYDVKDTAVTTKAYANQGTTLDVSGLDDAAIKAATGGTQGTASVTGGAVKFDADNNKYF VTIGGFTGADAAKNGDYEVNVATDGTVTLAAGATKTTMPAGATTKTEVQELKDTPAVVSADAKNALIAGGVDATDANGA ELVKMSYTDKNGKTIEGGYALKAGDKYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKTYQA SKAAGHDFKAQPELAEAAAKTTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNQLSEARSRIEDSDYATE VSQMSRAQILQQAGTSVLAQANQVPQNVLSLLRGSRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCV ADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA PATVCGPKKSTNLVKNKCVNFLEGSHHHHHH
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 -20ºC 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 4ºC for up to 1 week, -20ºC for up to 1 month or at -80ºC for up to 12 months. Avoid repeated freeze thaw cycles which may impact on protein activity.

Data

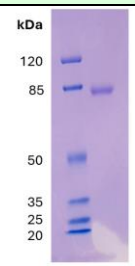

Figure 1: SDS PAGE analysis
1 µg of recombinant protein was separated by reducing SDS PAGE and visualised by Coomassie Blue staining. Caithness Biotech recombinant FljB-RBD migrates at approximately 84 kDa due to glycosylation.

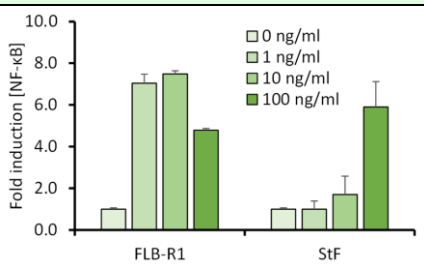

Figure 2: Validation of the capacity of recombinant FljB-RBD to stimulate TLR5-signalling
HEK-293 cells were transfected with NF-κB reporter and TLR5, then treated with indicated concentrations of the reconstituted protein (FLB-R1) or native (non-recombinant) *S. typmimurium* flagellin (StF) overnight. 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].

The spike (S) protein of the SARS-CoV-2 virus, which is responsible for the respiratory illness referred to as COVID-19, is essential for the virus's ability to infect human cells [5]. Specifically, the receptor-binding domain (RBD) of the spike protein is responsible for directly interacting with the angiotensin-converting enzyme 2 (ACE2) receptor on target cells, enabling virus attachment and entry. Being prominently exposed on the surface of the viral particle, the RBD is also a dominant antigen of the humoral immune response to SARS-CoV-2 infection. For these reasons, it has become the most commonly targeted antigen for the generation of candidate and approved vaccines, and for use in studies of serological conversion [6,7].

Caithness Biotech recombinant full-length *Salmonella enterica* serovar Typhimurium flagellin with C-terminal SARS-CoV2 RBD domain comprises amino acids Met 1 to Arg 506 of *S.* Typhimurium FljB protein, followed by amino acids Arg 319 to Phe 541 of the spike protein of the original SARS-CoV-2 viral sequence, first reported in January 2020 (Wuhan-Hu-1, Pango lineage A, Nextstrain Clade 19A [5]) fused to the C-terminus. This product retains potent TLR5 stimulatory capacity, and is 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 flagellin fusion proteins include use in studies of innate immune signalling, and for vaccine development.

References

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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)
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