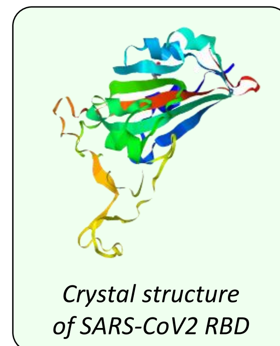
Caithness
BIOTECHNOLOGIES

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

SARS-CoV2 receptor binding domain protein

Background

The SARS-CoV-2 virus, which is responsible for the respiratory illness referred to as COVID-19, infects human cells by means of interaction between the viral spike (S) protein and the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of target cells. The receptor-binding domain (RBD) is the specific region of the spike protein that is responsible for this interaction, enabling virus attachment and entry [1]. 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 SARS-CoV-2 vaccines, and for use in studies of serological conversion following vaccination or natural infection [2,3].



Crystal structure of SARS-CoV2 RBD

Caithness Biotech RBD protein comprises 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 [1]). Potential applications of RBD protein include use in vaccine development, serological assays (such as ELISAs to measure RBD-specific antibody responses), drug discovery for inhibition of ACE2 binding and use in bioassays to explore other potential biological activities of the protein.

Advantages of mammalian cell expression

Recombinant antigens expressed in bacterial systems, such as *Escherichia coli*, frequently lack the correct folding and disulphide bond formation necessary to properly adopt the antigenic structure seen in the equivalent protein expressed in mammalian cells. Likewise, prokaryote and yeast systems fail to glycosylate recombinant proteins in the same way they are in human cells. Thus, mammalian cell expression is preferred for the expression of many recombinant antigens, including those of viruses that naturally infect human cells. Our RBD protein is expressed in HEK-293 cells for authentic glycosylation and antigenic structure, with very low levels of contamination with bacterial PAMPs, such as lipopolysaccharide (LPS, endotoxin).

Proven antigen specificity and economic format

We confirm correct antigenic structure of our RBD proteins by ELISA using RBD-binding monoclonal antibodies (see example shown in Figure 1). Each vial contains 100 µg of lyophilised protein. As RBD ELISA protocols often stipulate a coating density of 0.1 µg RBD antigen per well of a 96-well plate, one vial of our RBD protein is sufficient to prepare 10x 96-well plates for ELISA testing of RBD seropositivity or antibody binding.

<u>Key advantages of our recombinant proteins</u>

All of our proteins are expressed in mammalian cells for:

- ✓ Extremely low levels of non-specific PAMP contaminants, validated by HEK-293 TLR2 / TLR4 bioassays
- ✓ Human glycosylation patterns for authentic structure and antigenicity
- ✓ Superior folding and disulphide bond formation in comparison to proteins expressed in *E. coli*

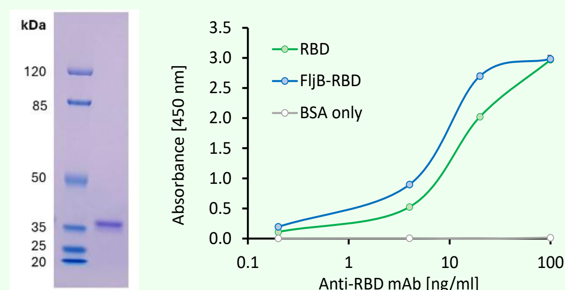


Figure 1: Typical SDS PAGE analysis of RBD protein and ELISA to confirm antigenic specificity of the protein. 96-well plates were coated with 0.1 µg/well of RBD antigen only, or flagellin (FljB)-RBD fusion protein overnight, then blocked with BSA and probed with indicated doses of RBD targeting mAb.

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SARS-CoV2 receptor binding domain protein

Also available as a flagellin fusion protein

Flagellin is the principal structural protein of bacterial flagella, the helical appendages that enable bacterial motility [4]. 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 [5]. 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 [6]. 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 [7].

Caithness Biotech offers RBD covalently fused to recombinant flagellin proteins, for studies of innate immune signalling, host-pathogen interactions, immunoassays, and as an adjuvant and carrier for vaccine development (Figure 2). Uniquely, our flagellin fusion 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. Please visit our Product pages online for further information.

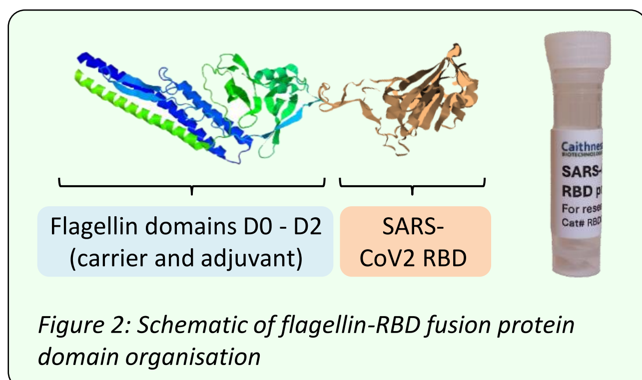

Figure 2: Schematic of flagellin-RBD fusion protein domain organisation

Low PAMP content for reliable investigation

Many reports have highlighted the benefit of utilising proteins with low levels of PAMP contamination when inflammatory signalling may confound the results. For example, numerous mammalian proteins, including heat-shock proteins (HSP) 60 and HSP-70, high mobility group box-1 (HMGB1) and beta-amyloid peptide, were reported to promote inflammation by directly stimulating TLR2 and/or TLR4 [8]. However, when low PAMP expression systems were used for their production, subsequent studies found that these proteins had no capacity to stimulate TLR-signalling [9-12].

We take three main approaches to minimise the PAMP content of our recombinant proteins: (i) expression in mammalian cells, (ii) stringent use of methods that prevent the re-introduction of PAMPs to the product during purification and (iii) continuous testing of reagents and products for PAMP content using the HEK-293 cell TLR transfection system to detect and quantify TLR2 and TLR4 stimulating contaminants (example shown in Figure 3).

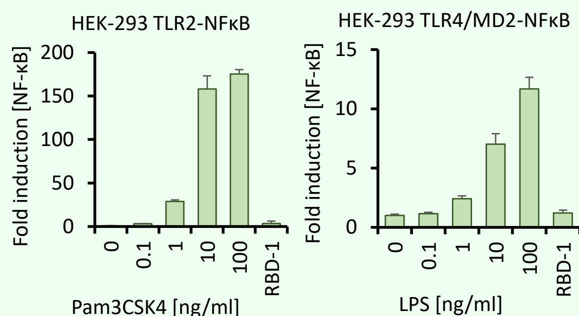

Figure 3: Example of TLR-assay confirming low levels of TLR2- and TLR4- stimulating contaminants in RBD protein. HEK-293 cells were transfected with NF-κB reporter and CD14 together with TLR2 or TLR4/MD2, then treated with indicated concentrations of Pam_3CSK_4 or E. coli LPS, or 1 µg/ml of reconstituted RBD protein. NF-κB signalling was measured after overnight treatment by luminometry.

References

[1] Zhou P, *et al.* Nature 579:270-273 (2020) [2] Kleanthous H, *et al.* npj Vaccines 6:128 (2021) [3] Amanat F, *et al.* Nature Medicine 26:1033-1036 (2020) [4] Samatey FA, et al. Nature 410:331-7 (2001) [5] Smith KD, et al. Nat Immunol 4:1247-53 (2003) [6] Zhao Y, et al. Nature 477:596-600 (2011) [7] Huleatt JW, et al. Vaccine 25:763-75 (2007) [8] Erridge C. J Leukoc Biol 87:989-99 (2010) [9] Gao B, Tsan MF. J Biol Chem 278:22523-22529 (2003) [10] Bausinger H, *et al.* Eur J Immunol 32:3708-3713 (2002) [11] Hreggvidsdottir HS, *et al.* J Leukoc Biol 86:655-662 (2009) [12] Youn JH, *et al.* J Immunol 180:5067-5074 (2008)

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