Recombinant SARS-CoV2 RBD protein

Description	
Product	Recombinant SARS-CoV2 Spike protein Receptor Binding Domain (RBD)
Catalogue number	RBD-01
Size / volume	100 µg
Expression system	HEK-293 cells
Amino acids	Arg 319 to Phe 541, NCBI reference sequence YP_009724390.1
Tags	C-terminal 6x His tag
Sequence graphic	SARS-CoV2 Spike RBD amino acids 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 ~ 38 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	RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYA DSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAG STPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFGSHHHHHH
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 200 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.
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

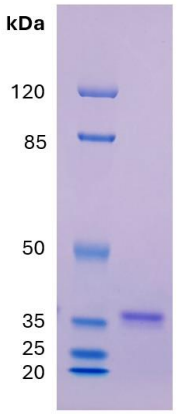

Figure 1: SDS PAGE analysis
1 µg of recombinant protein was separated by reducing SDS PAGE and visualised by Coomassie Blue staining. Caithness Biotech recombinant SARS-CoV2 RBD migrates at approximately 38 kDa due to glycosylation.

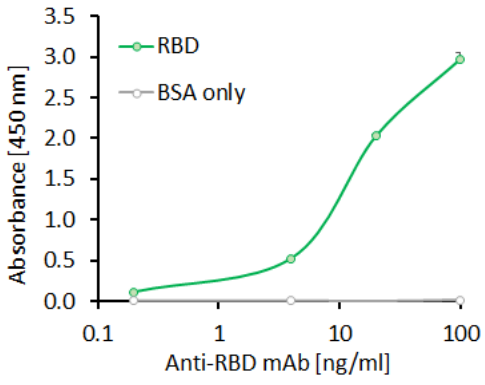

Figure 2: Validation of RBD mAb binding by ELISA
RBD protein was plated at 0.1 µg per well of a high binding 96-well plate and allowed to bind overnight. The binding of a SARS-Cov2 RBD specific monoclonal antibody at indicated concentrations was then measured by ELISA. Binding of the mAb to BSA only is shown as a negative control.

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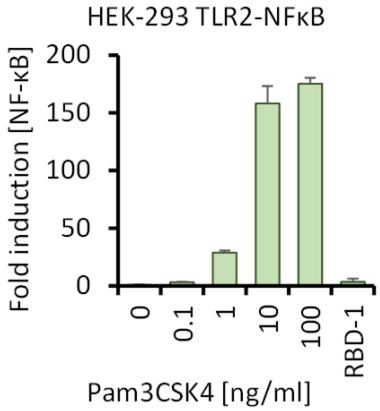

Figure 3: Validation of low levels of TLR2 stimulating contaminants
HEK-293 cells were transfected with NF-κB reporter and CD14 together with TLR2, then treated with indicated concentrations of Pam_3CSK_4, or 1 µg/ml of reconstituted RBD protein. NF-κB signalling was measured after overnight treatment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

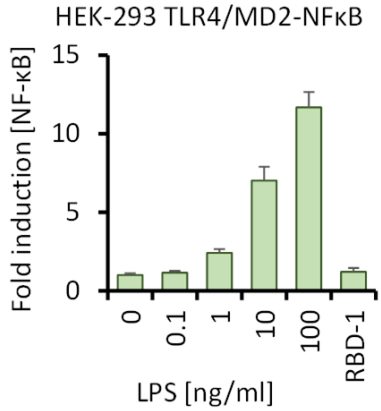

Figure 4: Validation of low levels of TLR4 stimulating contaminants
HEK-293 cells were transfected with NF-κB reporter and CD14 together with TLR4 and MD2, then treated with indicated concentrations of *E. coli* LPS, or 1 µg/ml of reconstituted RBD protein. NF-κB signalling was measured after overnight treatment by luminometry.

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 candidate and approved vaccines, and for use in studies of serological conversion following vaccination or natural infection [2,3].

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.

References

1)	Zhou P, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579:270-273 (2020)
2)	Kleanthous H, Silverman JM, Makar KW, Yoon I-K, Jackson N, Vaughn DW. Scientific rationale for developing potent RBD-based vaccines targeting COVID-19. npj Vaccines 6:128 (2021)
3)	Amanat F, *et al*. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nature Medicine 26:1033-1036 (2020)