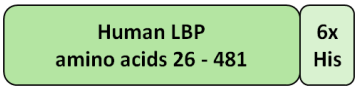
Recombinant human LPS binding protein

Description	
Product	Recombinant human LPS binding protein (LBP)
Catalogue number	LBP-1
Size / volume	20 µg
Expression system	HEK-293 cells
Amino acids	Ala 26 to Val 481, accession number P18428
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 ~ 63 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	AANPGLVARITDKGLQYAAQEGLLALQSELLRITLPDFTGDLRIPHVGRGRYEFHSLNIHSCELLHSALRPVPGQGLSL SISDSSIRVQGRWKVRKSFFKLQGSFDVSVKGISISVNLLLGSESSGRPTVTASSCSSDIADVEVDMSGDLGWLLNLFH NQIESKFQKVLESRICEMIQKSVSSDLQPYLQTLPVTTEIDSFADIDYSLVEAPRATAQMLEVMFKGEIFHRNHRSPVT LLAAVMSLPEEHNKMVYFAISDYVFNTASLVYHEEGYLNFSITDDMIPPDSNIRLTTKSFRPFVPRLARLYPNMNLELQ GSVPSAPLLNFSPGNLSVDPYMEIDAFVLLPSSSKEPVFRLSVATNVSATLTFNTSKITGFLKPGKVKVELKESKVGLF NAELLEALLNYYILNTFYPKFNDKLAEGFPLPLLKRVQLYDLGLQIHKDFLFLGANVQYMRVGSHHHHHH
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

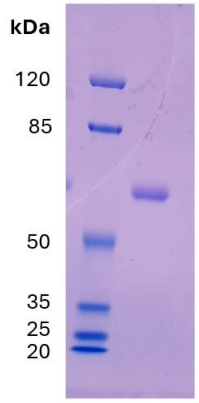


Figure 1: SDS PAGE analysis
1 µg of recombinant protein was separated by reducing SDS PAGE and visualised by Coomassie Blue staining. Caithness Biotech recombinant LBP migrates at approximately 63 kDa due to glycosylation.

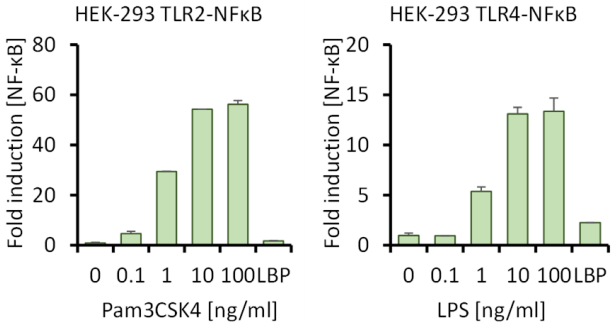


Figure 2: Validation of low levels of TLR2 and/or TLR4 stimulating contaminants
HEK-293 cells were transfected with NF-κB reporter and CD14 together with TLR2 or TLR4 and MD2, then treated with indicated concentrations of Pam_3CSK_4, or *E. coli* LPS, or the reconstituted protein at 1 µg/ml. NF-κB signalling was measured after overnight treatment by luminometry.

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Background

Lipopolysaccharide Binding Protein (LBP) is a soluble protein present in plasma that is produced mainly by hepatocytes in the liver. The primary function of LBP is to transfer monomers of lipopolysaccharide (LPS, endotoxin) from larger aggregates, such as Gram-negative bacterial cell membranes or outer membrane vesicles, to the CD14 receptor on monocytes and macrophages [1]. It is an acute phase protein, reaching relatively high concentrations in plasma (e.g. up to 100 µg/ml in man) during periods of systemic inflammatory insult. It is thought this may contribute to a negative feedback response to excessive inflammation induced by endotoxaemia, since high concentrations of LBP can limit inflammatory cytokine production in response to LPS [1,2]. It associates loosely in the circulation with various lipoprotein particles (such as high density lipoprotein), so modifying their capacity to sequester LPS from detection by pattern-recognition receptors [3].

Caithness Biotech recombinant LBP comprises amino acids Ala 26 to Val 481 of the native human sequence. Potential applications of LBP protein include use in bioassays to modify cellular sensitivity to LPS, studies of the capacity of LBP to transfer LPS and other lipids to various acceptors, and assays requiring monomeric display of LPS polysaccharides in a protein-bound form (e.g. as a capture reagent for ELISA).

References

1)	Gutsmann T, Müller M, Carroll SF, MacKenzie RC, Wiese A, Seydel U. Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. Infect Immun 69:6942-50 (2001)
2)	Thompson PA, Tobias PS, Viriyakosol S, Kirkland TN, Kitchens RL. Lipopolysaccharide (LPS)-binding protein inhibits responses to cell-bound LPS. J Biol Chem 278:28367-71 (2003)
3)	Han Y-H, Onufer EJ, Huang L-H, Sprung RW, Davidson WS, Czepielewski RS, Wohltmann M, Sorci-Thomas MG, Warner BW, Randolph GJ. Enterically derived high-density lipoprotein restrains liver injury through the portal vein. Science 373:eabe6729 (2021)