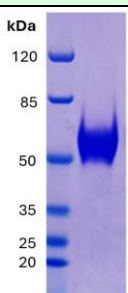
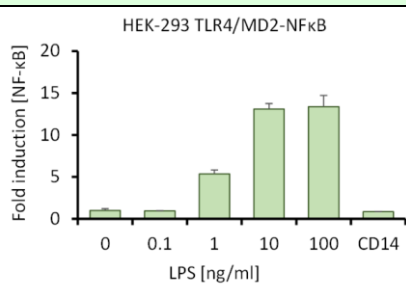


# Recombinant human CD14 protein

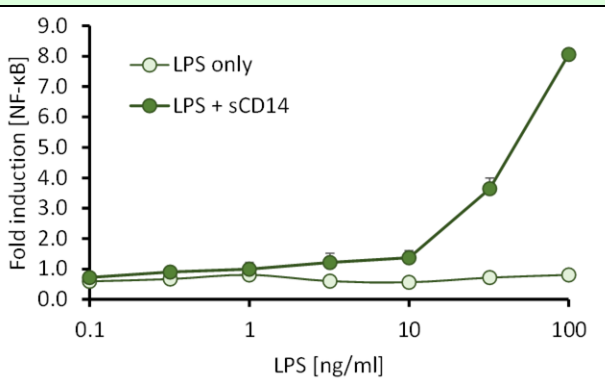
Description	
Product	Recombinant human CD14 protein
Catalogue number	CD14-1
Size / volume	20 µg
Expression system	HEK-293 cells
Amino acids	Thr 20 to Met 344, accession number P08571
Tags	C-terminal 6x His tag
Sequence graphic	<div style="display: flex; align-items: center; gap: 10px;"> <div style="border: 1px solid black; border-radius: 10px; padding: 5px; background-color: #e0f0e0;">Human CD14 amino acids 20 - 344</div> <div style="border: 1px solid black; border-radius: 10px; padding: 5px; background-color: #e0f0e0;">6x His</div> </div>
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 ~ 57 kDa (glycosylation present)
LPS content	< 0.1 ng / µg (by HEK-293-TLR4 bioassay, relative to <i>E. coli</i> LPS standard)
Amino acid sequence	ATTPEPCELDDDEDFRCVCFNFSEPPQPDWSEAFQCVSAVEVEIHAGGLNLEPFLKRVADADPRQYADTVKALRVRRLTVGAAQVPAQLLVGALRVLAYSRKELTLEDLKITGTMPPLPLEATGLALSSLRLRNVSATGRSWLAEQLQWLKPKGLKVLVIAQAHSPAFSCEQVRAFPALTSLDLSDNPLGERGLMAALCPHKFPALQNLALRNTGMETPTGVCAALAAAGVQPHSLDLSHNSLRATVNPSPAPRCMWSSALNSLNSLNSFAGLEQVPGKLPKLRVLDLSCNRLNRPQPDDELPEVDNLTLDGNPFLVPGTALPHEGSMGSHHHHHH
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	
	
<p><b>Figure 1: SDS PAGE analysis</b> 10 µg of recombinant CD14 was separated by reducing SDS PAGE and visualised by Coomassie Blue staining. Caithness Biotech recombinant CD14 migrates at approximately 57 kDa due to glycosylation.</p>	<p><b>Figure 2: Validation of low levels of TLR4 stimulating contaminants</b> HEK-293 cells were transfected with NF-κB reporter and CD14 together with TLR4 and MD2, then treated with indicated concentrations of <i>E. coli</i> LPS, or the reconstituted protein at 1 µg/ml. NF-κB signalling was measured after overnight treatment by luminometry.</p>

# Recombinant human CD14 protein

Data																									
 <table border="1"> <caption>Approximate data from Figure 3</caption> <thead> <tr> <th>LPS [ng/ml]</th> <th>Fold induction [NF-κB] (LPS only)</th> <th>Fold induction [NF-κB] (LPS + sCD14)</th> </tr> </thead> <tbody> <tr> <td>0.1</td> <td>~0.8</td> <td>~0.8</td> </tr> <tr> <td>0.5</td> <td>~0.8</td> <td>~0.9</td> </tr> <tr> <td>1</td> <td>~0.8</td> <td>~1.0</td> </tr> <tr> <td>5</td> <td>~0.8</td> <td>~1.2</td> </tr> <tr> <td>10</td> <td>~0.8</td> <td>~1.5</td> </tr> <tr> <td>30</td> <td>~0.8</td> <td>~3.8</td> </tr> <tr> <td>100</td> <td>~0.8</td> <td>~8.0</td> </tr> </tbody> </table>	LPS [ng/ml]	Fold induction [NF-κB] (LPS only)	Fold induction [NF-κB] (LPS + sCD14)	0.1	~0.8	~0.8	0.5	~0.8	~0.9	1	~0.8	~1.0	5	~0.8	~1.2	10	~0.8	~1.5	30	~0.8	~3.8	100	~0.8	~8.0	
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<p><b>Figure 3: Validation of capacity of sCD14 to enhance sensitivity of TLR4 signalling complex to LPS</b> HEK-293 cells were transfected with NF-κB reporter and TLR4/MD2, but without a construct for expression of membrane-bound CD14. Cells were then treated with indicated concentrations of <i>E. coli</i> LPS in the presence or absence of reconstituted recombinant sCD14 at 100 ng/ml. All treatments were in DMEM lacking serum (0% FCS). NF-κB signalling was measured after overnight treatment by luminometry.</p>																									

Background	
<p>CD14 acts as a co-receptor for the detection of bacterial lipopolysaccharide (LPS, endotoxin), which is a component of the outer membrane of Gram-negative bacteria [1]. CD14 is naturally expressed on the surface of certain immune cells, such as monocytes and macrophages, in a glycosylphosphatidylinositol (GPI) anchored form. CD14 also exists in plasma as a soluble form, lacking the GPI anchor (sCD14). Both membrane bound and soluble CD14 greatly enhance cellular sensitivity to LPS by promoting the transfer of LPS monomers from LPS-binding protein (LBP) to the TLR4/MD2 complex on the cell surface, which in turn triggers a signalling cascade that results in the production of inflammatory mediators. CD14 also enhances the sensitivity of cells to bacterial lipopeptides (BLP), by promoting the transfer of BLP to TLR2 [2].</p> <p>Caithness Biotech recombinant CD14 comprises amino acids Thr 20 to Met 344 of the native human sequence, so lacks the GPI anchor lipid which is attached to the membrane-bound form at asparagine 345. Potential applications of sCD14 protein include use in bioassays to enhance cellular sensitivity to LPS, studies of the capacity of CD14 to transfer BLP, LPS and other lipids to various acceptors, and assays requiring monomeric display of LPS polysaccharides in a protein-bound form.</p>	

References	
1)	Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. <i>Science</i> 249:1431-3 (1990)
2)	Nakata T, Yasuda M, Fujita M, Kataoka H, Kiura K, Sano H, Shibata K. CD14 directly binds to triacylated lipopeptides and facilitates recognition of the lipopeptides by the receptor complex of Toll-like receptors 2 and 1 without binding to the complex. <i>Cell Microbiol</i> 8:1899-909 (2006)