

**DESCRIPTION**

<b>Source</b>	Mouse myeloma cell line, NS0-derived human TLR4/MD2 Complex protein		
	Human TLR4 (Glu24-Lys631) Accession # O00206.2	S	10-His tag
	Human MD-2 (Glu17-Asn160) Accession # Q9Y6Y9.1	IEGRGGGSGGGSGGGG	10-His tag
	N-terminus		C-terminus
<b>N-terminal Sequence Analysis</b>	Glu24 (TLR4) and Glu17 (MD-2)		
<b>Predicted Molecular Mass</b>	70.6 kDa (TLR4), 19.2 kDa (MD-2)		

**SPECIFICATIONS**

<b>SDS-PAGE</b>	95 kDa and 34 kDa, reducing conditions
<b>Activity</b>	Measured by its ability to inhibit LPS-induced TNF- $\alpha$ secretion by PMA-differentiated U937 human histiocytic lymphoma cells. 20 $\mu$ g/mL of recombinant human TLR4/MD-2 will inhibit >60% of the TNF- $\alpha$ secretion induced by LPS.
<b>Endotoxin Level</b>	<1.0 EU per 1 $\mu$ g of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in PBS. See Certificate of Analysis for details.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 100 $\mu$ g/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 3 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

TLR4 is a 100 kDa type I transmembrane glycoprotein that belongs to the mammalian Toll-Like Receptor family of pathogen pattern recognition molecules. MD-2, also known as ESOP-1, is a 25 kDa secreted protein that is required for TLR4-mediated responses to bacterial lipopolysaccharide (LPS) (1 - 3). The human TLR4 cDNA encodes an 839 amino acid (aa) precursor that contains a 23 aa signal sequence, a 608 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 187 aa cytoplasmic domain. TLR4 contains 21 leucine rich repeats in its ECD and one cytoplasmic Toll/IL-1 receptor (TIR) domain (4). The ECD of human TLR4 shares approximately 25% aa sequence identity with other TLRs and 60% - 74% aa sequence identity with bovine, equine, feline, mouse, rat, and porcine TLR4. The human MD-2 cDNA encodes a 160 aa precursor with an 18 aa signal sequence (5). Human MD-2 shares 20% aa sequence identity with MD-1 and 62% - 64% aa sequence identity with bovine, mouse, and rat MD-2. MD-2 associates with TLR4 on monocytes, macrophages, dendritic cells, and B cells (5 - 7). MD2 expression is required for cell surface localization of TLR4 and for optimal LPS-induced TLR4 signaling (7, 8). MD-2 also forms soluble disulfide-linked homo-oligomers which can interact with TLR4 (6). Through a domain separate from its TLR4-binding domain, MD-2 extracts LPS from circulating CD14-LPS complexes and carries the LPS into a ternary complex with TLR4 (9 - 11). The interaction of MD-2/LPS with TLR4 induces receptor oligomerization and the triggering of an inflammatory response (12). Increased levels of plasma MD-2 in septic shock patients sensitizes MD-2 non-expressing epithelial cells to LPS and promotes widespread tissue inflammation (13).

**References:**

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