

Human CD14 protein, His tag (active)

Cat. No. GTX00249-pro

Applications	Functional Assay
Species	Human

Package

10 µg

Applications

Application Note

Cluster Of Differentiation 14 (CD14), also known as CD14, is a component of the innate immune system. CD14 acts as a co-receptor (along with the Toll-like receptor TLR 4 and MD-2) for the detection of bacterial lipopolysaccharide (LPS). CD14 can bind LPS only in the presence of lipopolysaccharide-binding protein (LBP). Although LPS is considered its main ligand, CD14 also recognizes other pathogen-associated molecular patterns such as lipoteichoic acid. Besides, Lipopolysaccharide Binding Protein (LBP) has been identified as an interactor of CD14, thus a binding ELISA assay was conducted to detect the interaction of recombinant human CD14 and recombinant human LBP. Briefly, CD14 were diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to LBP-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CD14 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 µl stop solution to the wells and read at 450nm immediately. The binding activity of CD14 and LBP was in a dose dependent manner.

CD14 also can enhance LPS-stimulated IL-8 secretion by THP-1 human acute monocytic leukemia cells. Therefore, THP-1 cells were cultured in 24 well plates at a concentration of 1×10^6 cells/ml and stimulated by LPS (1 µg/ml), then added of different concentrations of recombinant human CD14 (1 µg/ml, 5 µg/ml). After 24h later, the production of IL8 was determined in the supernatants by cytokine specific ELISA. When the concentration of CD14 was 5 µg/ml, the secretion LPS-stimulated IL-8 can be significantly increased.

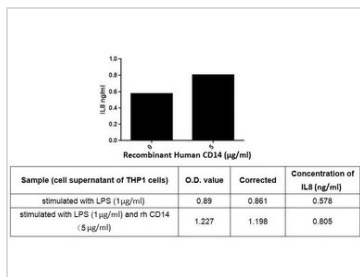
Observed MW (kDa)	18 kDa.
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Properties

Form	Lyophilized powder
Buffer	Reconstitute with 20mM Tris and 150mM NaCl to 0.1-1.0mg/ml. Do not vortex. Lyophilized from 20mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose.
Preservative	ProClin 300
Storage	For short-term storage (1-2 weeks), store at 4°C. For long-term storage, store at -20°C or below. After reconstitution, keep as concentrated solution. Avoid freeze-thaw cycles.
Region/Sequence	N-terminal His-Tag; Asp125~Leu288 (NP_000582.1)
Expression System	E. coli
Purity	> 97%
Endotoxin	< 1 EU/µg
Conjugation	Unconjugated
Note	For laboratory use only. Not for any clinical, therapeutic, or diagnostic use in humans or animals. Not for animal or human consumption.

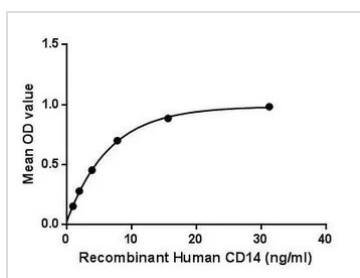


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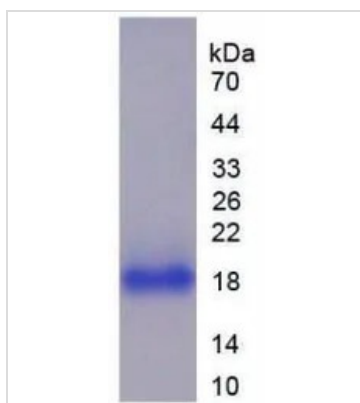
DATA IMAGES

GTX00249-pro Functional Assay Image

ELISA detection of secreted IL-8 protein activation from THP-1 cells treated with LPS (1µg/ml) and induced by GTX00249-pro Human CD14 protein (active) for 24hrs.

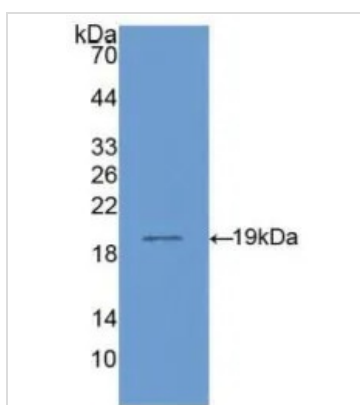
Recombinant protein concentrations: 5 µg/ml


GTX00249-pro Functional Assay Image

Functional ELISA analysis of GTX00249-pro Human CD14 protein (active) which can bind immobilized LBP protein.


GTX00249-pro Image

SDS-PAGE analysis of GTX00249-pro Human CD14 protein (active).


GTX00249-pro Image

WB analysis of GTX00249-pro Human CD14 protein (active).



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