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 37ºC. 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 37ºC. 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 $1x10^6$ cells/ml and stimulated by LPS (1µg/ml), then added of different concentrations of recombinant human CD14 (1µg/ml, 5µg/ml). After 24h later, the production of IL8 was determined in the supernatants by cytokine specific ELISA. When the concentration of CD14 was 5µg/ml, the secretion LPS-stimulated IL-8 can be significantly increased.

Observed MW (kDa)	18 kDa.

Properties

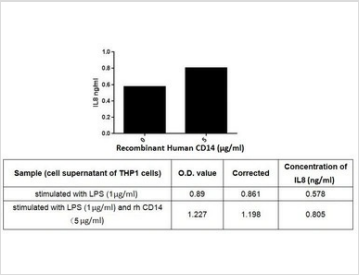
Form	Lyophilized powder
Buffer	Reconstitute with 20mM Tris and 150mM NaCl to 0.1-1.0mg/ml. Do not vortex. Lyophilized from 20mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose.
Preservative	ProClin 300
Storage	For short-term storage (1-2 weeks), store at 4ºC. For long-term storage, store at -20ºC 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.

For full product information, images and publications, please visit our website.

GeneTex, Inc. (North America)
1-877-436-3839 (Toll-free) 1-949-309-2888 sales@genetex.com

GeneTex International Corporation (Global)
886-3-6208988 886-3-6208989 infoasia@genetex.com

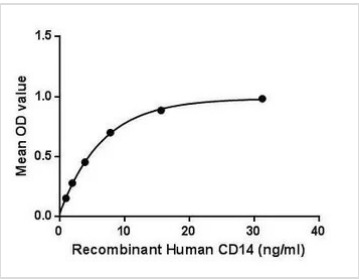
DATA IMAGES



Sample (cell supernatant of THP1 cells)	O.D. value	Corrected	Concentration of IL8 (ng/ml)
stimulated with LPS (1μg/ml)	0.89	0.861	0.576
stimulated with LPS (1μg/ml) and rh CD14 (5μg/ml)	1.227	1.198	0.805

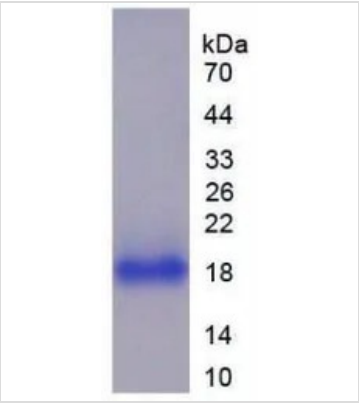
GTX00249-pro Functional Assay Image

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



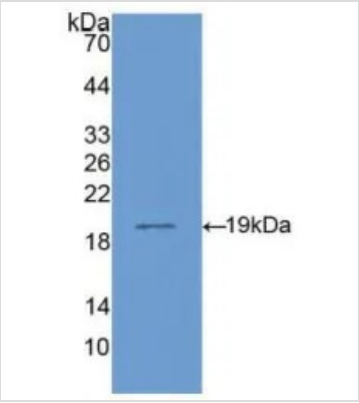
GTX00249-pro Functional Assay Image

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).

For full product information, images and publications, please visit our website.

GeneTex, Inc. (North America)
1-877-436-3839 (Toll-free) 1-949-309-2888 sales@genetex.com

GeneTex International Corporation (Global)
886-3-6208988 886-3-6208989 infoasia@genetex.com