

APA685Hu01 100µg

Active Cluster Of Differentiation 14 (CD14)

Organism Species: *Homo sapiens* (Human)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Asp125~Leu288

Tags: N-terminal His-tag

Purity: >98%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 8.5

Predicted Molecular Mass: 18.6kDa

Accurate Molecular Mass: 18kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

```
DLKITG TMPPLPLEAT GLALSSLRLR
NVSWATGRSW LAELQQWLKP GLKVLISIAQA HSPAFCSEQV RAFFALTSLD
LSDNPGGLGER GLMAALCPHK FPAIQNLALR NTGMETPTGV CAALAAAGVQ
PHSLDLSHNS LRATVNPSAP RCMWSSALNS LNLSFAGL
```

[ACTIVITY]

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 100µL 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 50µL stop solution to the wells and read at 450nm immediately. The binding activity of CD14 and LBP was shown in Figure 1, and this effect was in a dose dependent manner.

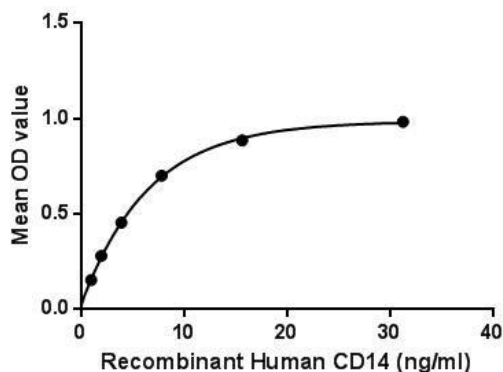


Figure 1. The binding activity of CD14 with LBP.

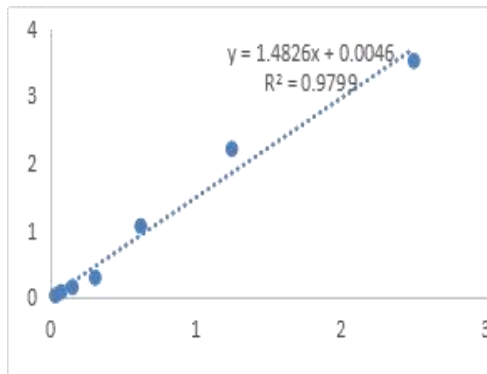
CD14 also can enhance LPS-stimulated IL-8 secretion by THP-1 human acute monocytic leukemia cells. Therefore, THP1 cells were cultured in 24 well plates at a concentration of 10^6 cells/mL and stimulated by LPS ($1\mu\text{g/mL}$), then added of different concentrations of recombinant human CD14 ($1\mu\text{g/mL}$, $5\mu\text{g/mL}$). After 24h later, the production of IL8 was determined in the supernatants by cytokine specific ELISA.

Result: When the concentration of CD14 was $5\mu\text{g/mL}$, the secretion LPS-stimulated IL-8 can be significantly increased. The results shown in table 1 and Figure 2.

Table 1. Effects of CD14 on IL8 production by THP1 cells.

Sample (cell supernatant of THP1 cells)	O.D. value	Corrected	Concentration of IL8 (ng/mL)
stimulated with LPS ($1\mu\text{g/mL}$) and rh CD14 ($5\mu\text{g/mL}$)	1.227	1.198	0.805
stimulatedwith LPS ($1\mu\text{g/mL}$) and rh CD14 ($1\mu\text{g/mL}$)	0.915	0.886	0.594
stimulatedwith LPS ($1\mu\text{g/mL}$)	0.89	0.861	0.578

The standard curve was demonstrated as follows:



STD (ng/mL)	O.D. value	Corrected
2.5	3.556	3.526
1.25	2.245	2.215
0.625	1.097	1.067
0.3125	0.329	0.299
0.15625	0.188	0.158
0.078125	0.117	0.087
0.0390625	0.065	0.035

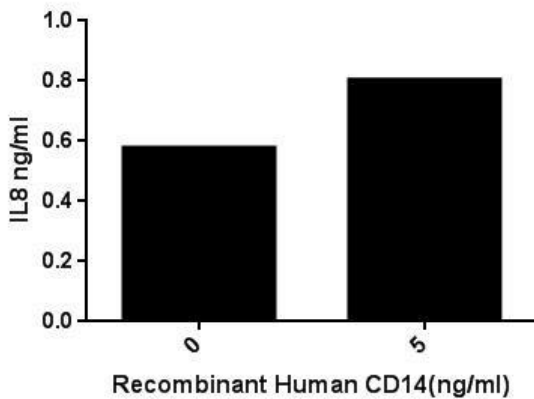


Figure 2. Effects of CD14 on IL8 production by THP1 cells.

[IDENTIFICATION]

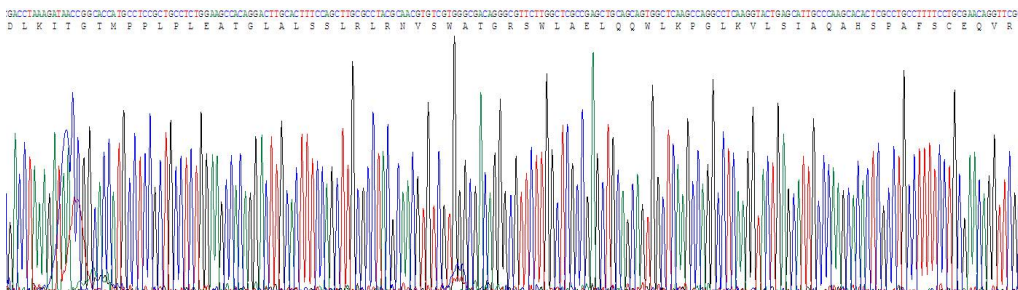


Figure 3. Gene Sequencing (extract)

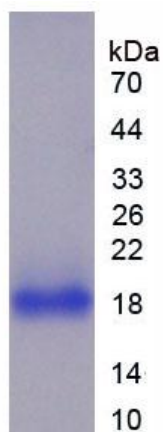


Figure 4. SDS-PAGE

Sample: Active recombinant CD14, Human

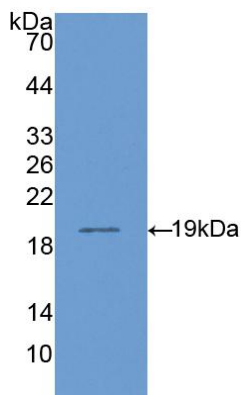


Figure 5. Western Blot

Sample: Recombinant CD14, Human;

Antibody: Rabbit Anti-Human CD14 Ab (PAA685Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.