Cloud-Clone Corp.

APA781Hu01 100µg Active Cluster Of Differentiation 276 (CD276) Organism Species: Homo sapiens (Human) Instruction manual

#### FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

### [PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Thr47~Ser219

Tags: N-terminal His-tag

**Purity: >98%** 

**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: 5.9

Predicted Molecular Mass: 22.4kDa

Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

## [<u>USAGE</u>]

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.

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**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.

### [<u>SEQUENCE</u>]

TLCC SFSPEPGFSL AQLNLIWQLT DTKQLVHSFA EGQDQGSAYA NRTALFPDLL AQGNASLRLQ RVRVADEGSF TCFVSIRDFG SAAVSLQVAA PYSKPSMTLE PNKDLRPGDT VTITCSSYQG YPEAEVFWQD GQGVPLTGNV TTSQMANEQG LFDVHSILRV VLGANGTYS

### [ACTIVITY]

Cluster Of Differentiation 276 (CD276) also known as B7-H3 in human, is a member of the B7/CD28 superfamily of costimulatory molecules serving as an accessory modulator of T-cell response. B7 proteins are immunoglobulin (Ig) superfamily members with extracellular Ig-V-like and Ig-C-like domains and short cytoplasmic domains. Among the family members, they share about 20-40% amino acid (aa) sequence identity. CD276 may play a protective role in tumor cells by inhibiting natural-killer mediated cell lysis as well as a role of marker for detection of neuroblastoma cells. It has also been found to enhance the induction of primary cytotoxic T lymphocytes and stimulate IFN-gamma production. Besides, Sialic acid-binding Ig-like lectin 12 (SIGLEC12) has been identified as an interactor of CD276, thus a binding ELISA assay was conducted to detect the interaction of recombinant human CD276 and recombinant human SIGLEC12. Briefly, CD276 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to SIGLEC12-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CD276 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  $37^{\circ}$ C. Finally, add  $50\mu$ L stop solution to the wells and read at 450nm immediately. The binding activity of CD276 and SIGLEC12 was shown in Figure 1, and this effect was in a dose dependent manner.

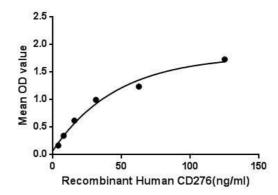


Figure 1. The binding activity of CD276 with SIGLEC12.

### [IDENTIFICATION]

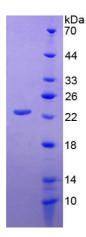


Figure 2. SDS-PAGE

Sample: Active recombinant CD276, Human

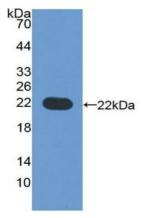


Figure 3. Western Blot

Sample: Recombinant CD276, Human;

Antibody: Rabbit Anti-Human CD276 Ab (PAA781Hu01)