APA670Hu61 100µg Active Homing Associated Cell Adhesion Molecule (HCAM) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: GIn21~Pro220

Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1

Purity: >90%

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

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 4.3

Predicted Molecular Mass: 49.0kDa

Accurate Molecular Mass: 85kDa as determined by SDS-PAGE reducing conditions. Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.

2. Relative charge: The composition of amino acids may affects the charge of the protein.

3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.

4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.

5. Polymerization of the target protein: Dimerization, multimerization etc.

[<u>USAGE</u>]

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Reconstitute in 10mM PBS (pH7.4) 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.

[<u>SEQUENCE</u>]

QIDLNITCRF AGVFHVEKNG RYSISRTEAA DLCKAFNSTL PTMAQMEKAL SIGFETCRYG FIEGHVVIPR IHPNSICAAN NTGVYILTSN TSQYDTYCFN ASAPPEEDCT SVTDLPNAFD GPITITIVNR DGTRYVQKGE YRTNPEDIYP SNPTDDDVSS GSSSERSSTS GGYIFYTFST VHPIPDEDSP WITDSTDRIP

[ACTIVITY]

Homing Associated Cell Adhesion Molecule (HCAM), also known as CD44, is a ubiquitous multistructural and multifunctional cells surface adhesion molecule involved in cell-cell and cell-matrix interactions. CD44 is broadly expressed, including in the membranes of B cells, granulocytes, monocytes, and erythrocytes as well as on many thymocytes and mature T cells, besides it is highly expressed in many cancers and regulates metastasis via recruitment of CD44 to the cell surface. This protein is a receptor for hyaluronic acid (HA) and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs). Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human HCAM and biotinylated hyaluronan (HA). Briefly, biotin-linked HA was diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to HCAM-coated microtiter wells and

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incubated for 2h at 37 $^\circ$ C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 1 hour, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^\circ$ C. Finally, add 50µl stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant human HCAM and biotinylated HA was shown in Figure 1, and this effect was in a dose dependent manner.

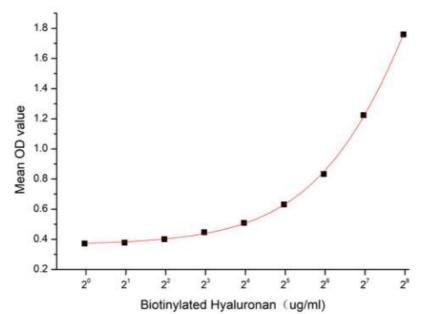
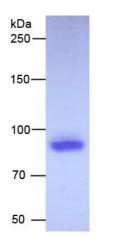


Figure 1. The binding activity of recombinant human HCAM and biotinylated HA



[IDENTIFICATION]

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Figure 2. SDS-PAGE

Sample: Active recombinant HCAM, Human

[<u>IMPORTANT NOTE</u>]

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