APA670Ra01 100µg Active Homing Associated Cell Adhesion Molecule (HCAM) Organism Species: *Rattus norvegicus (Rat) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Gln24~Val182 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 4.5 Predicted Molecular Mass: 21.4kDa Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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.

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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.

[SEQUENCE]

QIDLNIT CRYAGVFHVE KNGRYSISRT EAADLCEAFN TTLPTMAQME LALRKGFETC RYGFIEGHVV IPRIHPNAIC AANNTGVYIL LASNTSHYDT YCFNASAPLE EDCTSVTDLP NSFDGPVTIT IVNRDGTRYS KKGEYRTHQE DIDASNIIDE DV

[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 rat HCAM and biotinylated hyaluronan (HA). Briefly, biotin-linked HA were 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 incubated for 2h at 37 °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 °C . Finally, add 50 µl stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant rat HCAM and biotinylated HA

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was shown in Figure 1, and this effect was in a dose dependent manner.



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

[IDENTIFICATION]

	kDa 70
	44
	33
	26
-	22
	18
	14
	10



Sample: Active recombinant HCAM, Rat

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[<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.