APC463Hu01 500µg Active Endothelial Cell Specific Molecule 1 (ESM1) 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: Prokaryotic expression. Host: E. coli Residues: Trp20~Arg184 Tags: N-terminal His-tag **Purity:** >92% **Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose. Original Concentration: 1000µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.7 Predicted Molecular Mass: 19.4kDa Accurate Molecular Mass: 19&44kDa 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.

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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>]

W SNNYAVDCPQ HCDSSECKSS PRCKRTVLDD CGCCRVCAAG RGETCYRTVS GMDGMKCGPG LRCQPSNGED PFGEEFGICK DCPYGTFGMD CRETCNCQSG ICDRGTGKCL KFPFFQYSVT KSSNRFVSLT EHDMASGDGN IVREEVVKEN AAGSPVMRKW LNPR

[ACTIVITY]

Endothelial cell-specific molecule 1 (ESM1) is a proteoglycan secreted by endothelial cells (primarily in the human lung and kidney tissues) and its mRNA expression is regulated by inflammatory cytokines. Endocan expression, which detected in various epithelia and adipocytes has been shown to be upregulated by vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), TNF alpha, IL1 beta, or lipopolysaccharide and downregulated by IFN gamma. Genetically engineered cells overexpressing ESM1 induce tumor formation, implying that ESM1 might be involved in the pathophysiology of tumor growth in vivo. Besides, Lymphocyte Function Associated Antigen 1 Alpha (LFA1a) has been identified as an interactor of ESM1, thus a binding ELISA assay was conducted to detect the interaction of recombinant human ESM1 and recombinant human LFA1a. Briefly, ESM1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to LFA1a-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti- ESM1 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 50μ L stop solution to the wells and read at 450nm immediately. The binding activity of of ESM1 and LFA1a was shown in Figure 1, and this effect was in a dose dependent manner.







Figure 2. Gene Sequencing (extract)

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Figure 3. SDS-PAGE

Sample: Active recombinant ESM1, Human

[IMPORTANT NOTE]

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.