APC034Ra01 100µg Active Growth Differentiation Factor 15 (GDF15) 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: Ser189~Ala303 Tags: N-terminal His-tag Purity: >97% 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: 6.9 Predicted Molecular Mass: 13.9kDa Accurate Molecular Mass: 16kDa 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]

SAHLHPRDSCPLGPGRCCHLETVQATLEDLGWSDWVLSPRQLQLSMCVGECPHLYRSANTHALIKARLH GLQPDRVPAPCCVPSSYTPVVLMHRTDSGVSLQTYDDLVAQGCHCA

[ACTIVITY]

Growth Differentiation Factor 15 (GDF-15), also called Macrophage Inhibitory Cytokine 1 (MIC-1), is a divergent member of the Transforming Growth Factor beta (TGF-beta) superfamily. Human GDF-15 shares 66% and 68% amino acid sequence identity with the rat and mouse proteins, respectively. GDF-15 is highly expressed in placenta and brain, and it is expressed at lower levels in kidney, pancreas, prostate, and colon. Similar to other TGF-beta family proteins, GDF-15 is synthesized as a large precursor protein that is cleaved at a dibasic cleavage site (RxxR) to release the mature protein. Biologically active GDF-15 is a disulfide-linked homodimer of the mature protein. GDF-15 has been shown to have various functions, including inhibition of Tumor Necrosis Factor alpha (TNF-alpha) production from lipopolysaccharide-stimulated macrophages and the induction of cartilage formation. A functional binding ELISA assay was conducted to detect the interaction of recombinant rat GDF-15 and recombinant human GP9. Briefly, GDF-15 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to GP9-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-GDF-15 pAb, then aspirated and washed 3 times.

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After incubation with HRP labelled secondary antibody for 1h at 37 $^{\circ}$ C, 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 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of GDF-15 and GP9 was shown in Figure 1, the EC50 for this effect is 0.47 ug/mL.



Figure 1. The binding activity of recombinant rat GDF-15 and recombinant human GP9

[IDENTIFICATION]





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Figure 3. SDS-PAGE

Sample: Active recombinant GDF15, Rat

[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.