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APC299Mu01 100µg Active Apolipoprotein M (APOM) Organism Species: *Mus musculus (Mouse) 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: Met20~Lys190 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: 5.9 Predicted Molecular Mass: 25.8kDa Accurate Molecular Mass: 32kDa 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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[<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.

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]

M NQCPEHSQLT ALGMDDTETP EPHLGLWYFI AGAASTTEEL ATFDPVDNIV FNMAAGSAPR QLQLRATIRT KSGVCVPRKW TYRLTEGKGN MELRTEGRPD MKTDLFSSSC PGGIMLKETG QGYQRFLLYN RSPHPPEKCV EEFQSLTSCL DFKAFLVTPR NQEACPLSSK

[ACTIVITY]

Apolipoprotein M (APOM) is an approximately 25 kDa variably glycosylated protein that adopts a beta-barrel structure characteristic of lipocalin family proteins. It functions as a component of lipoprotein particles which play essential roles in fatty acid and cholesterol transport and metabolism. Alternative splicing generates a short isoform that lacks the N-terminal 72 amino acids. APOM is produced primarily by hepatocytes but also by renal tubule epithelial cells. The signal peptide is not cleaved and is required for APOM association with lipoprotein particles as well as Megalin mediated reabsorption by the kidney. The activity of recombinant mouse APOM was measured by its ability to bind all-trans retinoic acid. The binding of retinoic acid results in the quenching of Trp fluorescence in APOM. APOM was diluted to 50 ug/ml in 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, pH 7.5

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(assay buffer) and the retinoic acid was diluted to 400, 200, 100, 30, 10, 3, 1 and 0.5 μ M in 95% ethanol. Mixing 112.5 μ L of 50 μ g/mL rmAPOM and 12.5 μ L of retinoic acid serial dilutions in microtubes and a blank containing 112.5 μ L of 50 μ g/mL rmAPOM and 12.5 μ L of 95% ethanol, then incubate at room temperature for 30 minutes. Loading 100 ul of the reaction mixtures and blank and read at excitation and emission wavelengths of 280 nm and 340 nm (top read), respectively, in endpoint mode. The result was shown in figure 1, the 50% binding concentration (BC50) is >300 μ M.

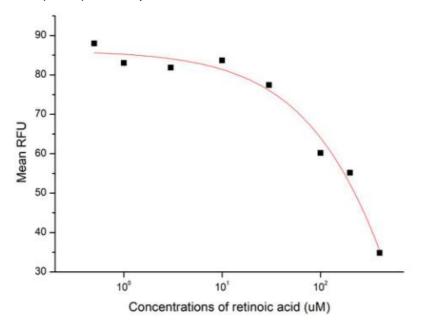
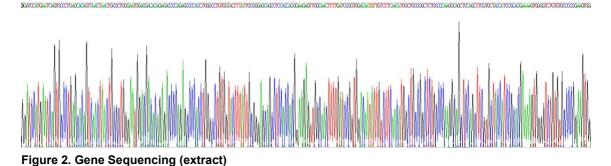


Figure 1 The binding activity of recombinant mouse APOM with retinoic acid

[IDENTIFICATION]



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	kDa 70
	44
-	33
	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant APOM, Mouse

[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.