APA929Mu01 100μg
Active Retinol Binding Protein 4 (RBP4)
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: Glu63~Leu245
Tags: N-terminal His-tag
Purity: >90%
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: 6.3
Predicted Molecular Mass: 21.4kDa
Accurate Molecular Mass: 19&22kDa 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.

[USAGE]

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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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]

ERDCRVSS FRVKENFDKA RFSGLWYAIA KKDPEGLFLQ
DNIIAEFSVD EKGHMSATAK GRVRLLSNWE VCADMVGTFT DTEDPAKFKM
KYWGVASFLQ RGNDDHWIID TDYDTFALQY SCRLQNLDGT CADSYSFVFS
RDPNGLSPET RRLVRQRQEE LCLERQYRWI EHNGYCQSRP SRNSL

[ACTIVITY]

Retinol-binding protein 4 (RBP4) is the specific carrier for retinol (also known as vitamin A), and is responsible for the conversion of unstable and insoluble retinol in aqueous solution into stable and soluble complex in plasma through their tight interaction. As a member of the lipocalin superfamily, RBP4 containing a β-barrel structure with a well-defined cavity is secreted from the liver, and in turn delivers retinol from the liver stores to the peripheral tissues. In plasma, the RBP4-retinol complex interacts with transthyretin (TTR), and this binding is crucial for preventing RBP4 excretion through the kidney glomeruli. RBP4 expressed from an ectopic source efficiently delivers retinol to the eyes, and its deficiency affects night vision largely. Recently, RBP4 as an adipokine, is found to be expressed in adipose tissue and correlated with obesity, insulin resistance (IR) and type 2

diabetes (T2DM). The activity of recombinant mouse RBP4 was measured by its ability to bind all-trans retinoic acid. The binding of retinoic acid results in the quenching of Trp fluorescence in RBP4. RBP4 was diluted to 50 ug/ml in 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, pH 7.5 (assay buffer) and the retinoic acid was diluted to 800, 400, 200, 100, 30, 10, 3, 1, 0.5 and 0.1 uM in 95% ethanol. Mixing 112.5 µL of 50 µg/mL rmRBP4 and 12.5 µL of retinoic acid serial dilutions in microtubes and a blank containing 112.5 µL of 50 µg/mL rmRBP4 and 12.5 µL 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 > 4.6 µM.

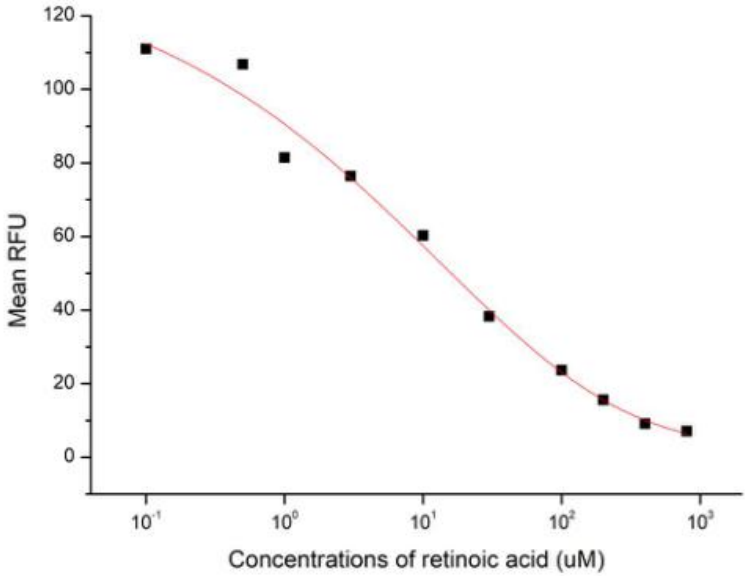

Figure 1 The binding activity of recombinant mouse RBP4 with retinoic acid

[IDENTIFICATION]

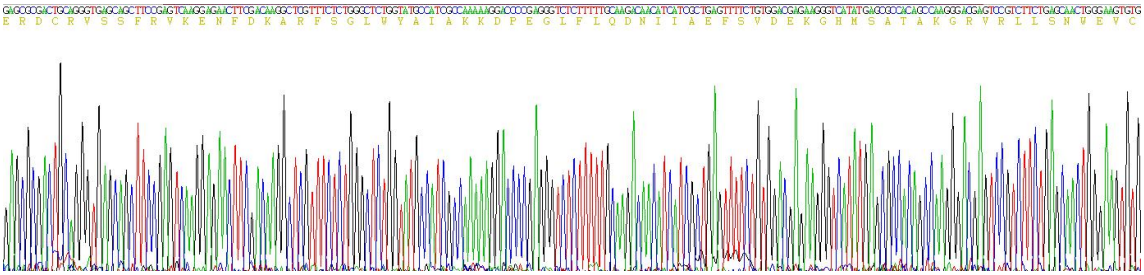

Figure 2. Gene Sequencing (extract)

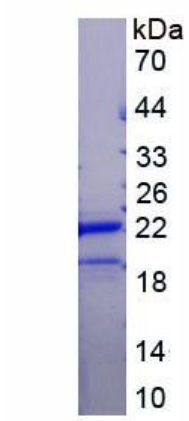

Figure 3. SDS-PAGE

Sample: Active recombinant RBP4, 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.