APA551Mu01 100μg
Active Fibroblast Growth Factor 2, Basic (FGF2)
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: Pro10~Ser154
Tags: N-terminal His-tag
Purity: >98%
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: 9.7
Predicted Molecular Mass: 20.0kDa
Accurate Molecular Mass: 20/23kDa 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]

P ALPEDGGAAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHVK LQLQAEERGV VSIKGVCANR YLAMKEDGRL LASKCVTEEC FFFERLESNN YNTYRSRKYS SWYVALKRTG QYKLGSKTGP GQKAILFLPM SAKS

[ACTIVITY]

Basic fibroblast growth factor (FGF2), also known as bFGF, FGF-β is a member of a large family of structurally related heparin-binding proteins (the FGFs) involved in the regulation of cell proliferation, growth and differentiation. It involved in many biological processes including angiogenesis, embryonic development and wound healing. Additionally, FGF2 is a critical component of human embryonic stem cell culture medium. Besides, Caspase 1 (CASP1) has been identified as an interactor of FGF2, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse FGF2 and recombinant mouse CASP1.

Briefly, FGF2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to CASP1-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-FGF2 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℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of FGF2 and CASP1 was shown in Figure 1, and this effect was in a dose dependent manner.

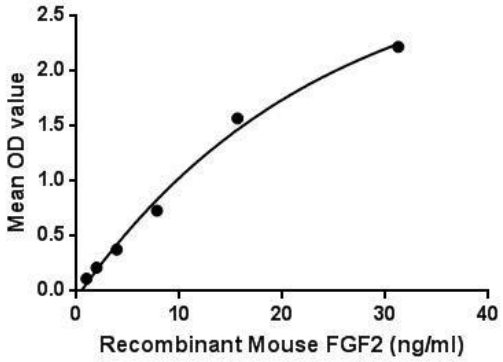

Figure 1. The binding activity of FGF2 with CASP1.

To test the effect of rmFGF2 on cell proliferation of 3T3 fibroblasts , Balb/c 3T3 cells were seeded into triplicate wells of 96-well plates at a density of 8, 000 cells/well and allowed to attach overnight, then the medium was replaced with 0.4% FBS standard DMEM prior to the addition of various concentrations of recombinant mouse FGF2. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μl of CCK-8 solution was added to each well of the plate，then measure the absorbance at 450 nm using a microplate reader after incubating the plate for 1-4 hours at 37 °C. Cell proliferation of Balb/c 3T3 cells after incubation with rhEGF for 48h observed by inverted microscope was shown in Figure 2. The dose-effect curve of rmFGF2 was shown in Figure 3. It was obvious that rmFGF2 significantly promoted cell proliferation of 3T3 cells. The ED50 for this effect is

typically 0.2 ug/ml.

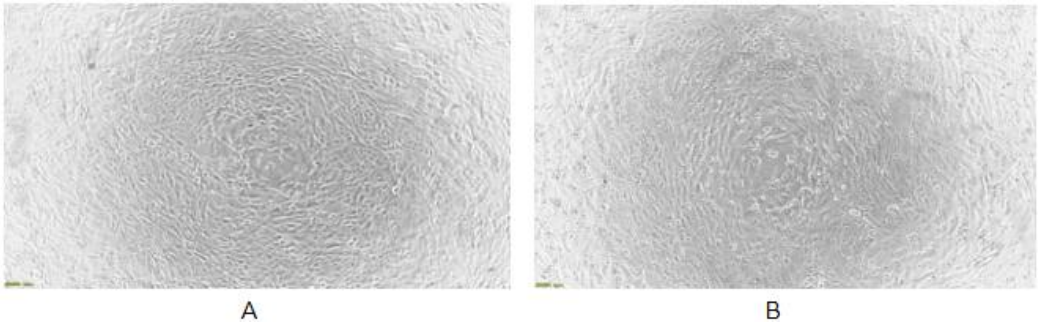

Figure 2. Cell proliferation of Balb/c 3T3 cells after stimulated with rmFGF2 .

(A) Balb/c 3T3 cultured in DMEM, stimulated with 1 ug/ml rmFGF2 48h;

(B) Unstimulated Balb/c 3T3 cells cultured in 0.4% FBS DMEM for 48h.

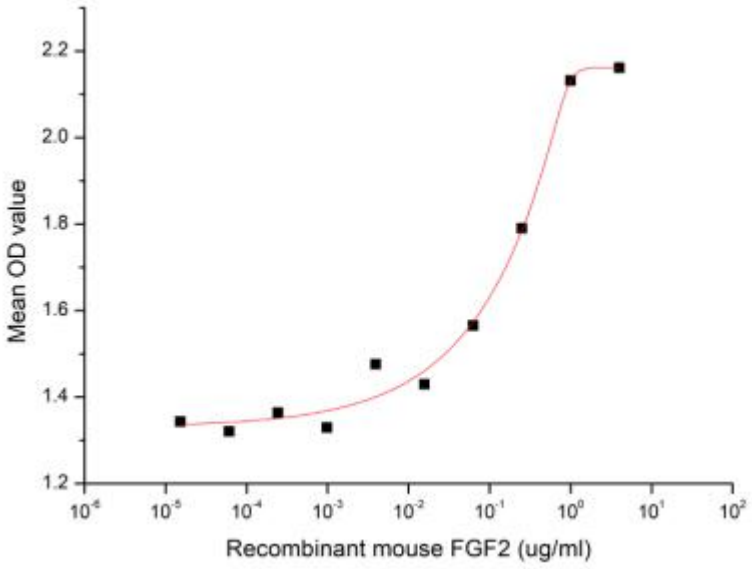

Figure 3. The dose-effect curve of recombinant mouse FGF2 on Balb/c 3T3 cells

[IDENTIFICATION]

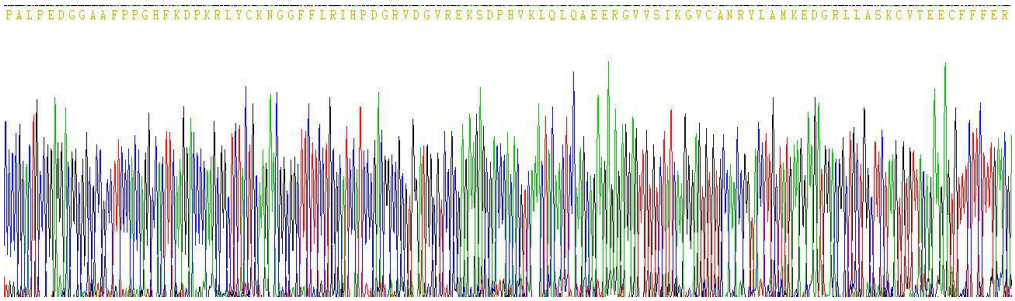

Figure 4. Gene Sequencing (extract)

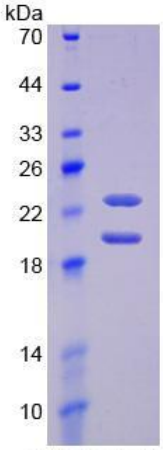

Figure 5. SDS-PAGE

Sample: Active recombinant FGF2, 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.