APK541Mu01 100μg
Active Regenerating Islet Derived Protein 3 Beta (REG3b)
Organism Species: Mus musculus (Mouse)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Glu27~Gly175
Tags: N-terminal His-tag
Purity: >99%
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 7.8
Predicted Molecular Mass: 20.4kDa
Accurate Molecular Mass: 17kDa 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 20mM Tris, 150mM NaCl (pH8.0) 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.

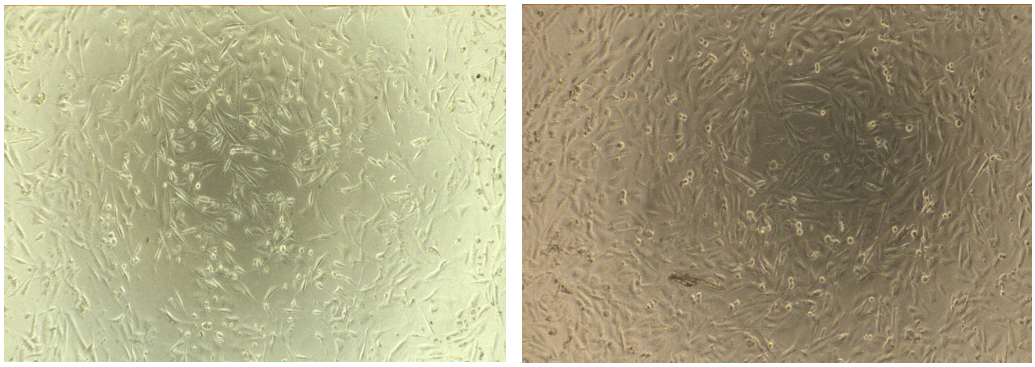
[SEQUENCE]

EDSL KNIPSARISC PKGSQAYGSY
CYALFQIPQT WFDAELACQK RPGGHLVSVL NSAEASFLSS MVKRTGNSYQ
YTWIGLHDPT LGAEPNGGGW EWSNNDVMNY FNWERNPSTA LDRAFCGSLS
RASGFLKWRD MTCEVKLPYV CKFTG

[ACTIVITY]

Regenerating Islet Derived Protein 3 Beta (REG3b) also known as PAP-I and HIP is bactericidal C-type lectin which acts against several intestinal Gram-positive bacteria and Gram-negative bacteria. The Reg family proteins have been implicated in a range of physiological processes including acting as acute phase reactants, lectins, survival/growth factors for insulin-producing pancreatic beta-cells, neural cells, and epithelial cells of the digestive system. To test the effect of REG3b on cell proliferation of SK-N-SH, SK-N-SH cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of REG3b. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10μL of CCK-8 solution was

added to each well of the plate, then measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37°C .Cell proliferation of SK-N-SH cells after incubation with REG3b for 72h observed by inverted microscope was shown in Figure 1.



A B

Figure 1. Cell proliferation of SK-N-SH cells after stimulated with REG3b.

(A) Unstimulated SK-N-SH cells cultured in serum-free DMEM for 72h.

(B) SK-N-SH cells cultured in DMEM, stimulated with 10ng/mL REG3b 72h;

The dose-effect curve of REG3b was shown in Figure 2. It was obvious that REG3b significantly promoted cell proliferation of SK-N-SH cells. The ED50 for this effect is typically3.685-84.37ng/mL.

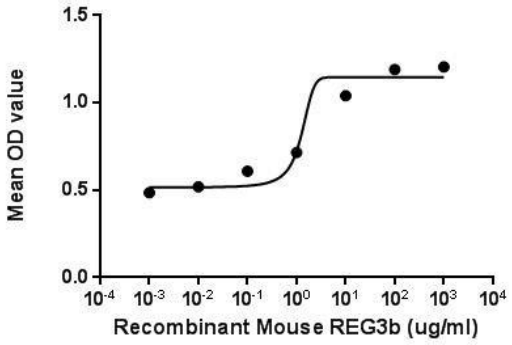


Figure 2. The dose-effect curve of REG3b on SK-N-SH cell.

[IDENTIFICATION]

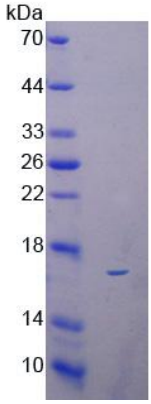


Figure 3. SDS-PAGE

Sample: Active recombinant REG3b, Mouse

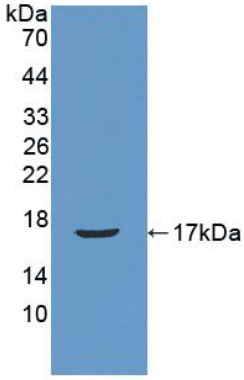


Figure 4. Western Blot

Sample: Recombinant REG3b, Mouse;

Antibody: Rabbit Anti-Mouse REG3b Ab (PAK541Mu01)