Human IL-32 ELISA Kit

Catalogue Number: SEL101

ORDERING INFORMATION

Components
Capture Antibody 40 ug
Detection Antibody 10 ug
Standard protein 100 ng

Formulation: Lyophilized powder

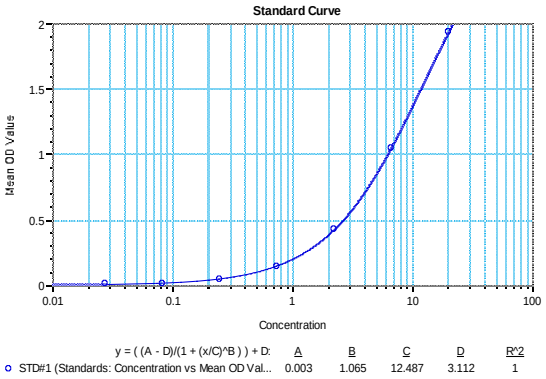
Storage: -20℃

Reconstitution or dilution: PBS
Standard protein: Add 100 ul PBS (1 ug/ml, 50x)
Specificity: human IL-32

Recommended Usage:
Capture antibody: 2 ug/ml in PBS
Detection antibody: 0.5 ug/ml in PBS
Standard protein: 20 ng/ml, 3-fold

General protocol of ELISA:
Coat 100 ul of the capture antibody on a 96-well immunoplate overnight at 4˚C or for 2 hours at room temperature. Block the plate with 200 ul of 3% albumin containing PBS for 2 hours at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add samples to detect IL-32 or standard protein overnight at 4˚C or for 2 hours at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add 100 ul of the detection antibody for 2 hours at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add 100 ul of HRP-conjugated anti-rabbit Ab for 1 hour at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add 100 ul of tetramethyl benzidine (TMB) solution until the standard wells turn to blue. This may not take more than 20 minutes. Add another 100 ul of 1N H_2SO_4 and read the optical density at 450 nm wave.

Standard Curve



IL-32 (ng/ml)	OD values
blank	0.0567
20	1.9968
6.7	1.1123
2.2	0.4896
0.74	0.2049
0.25	0.1041
0.082	0.0702

Background

Interleukin-32 gamma (IL-32γ), a proinflammatory cytokine in previous term, natural killer cells transcript 4 (NK4) or tumor necrosis factor alpha (TNFα) inducing factor, is a 27 kDa, secretory glycoprotein. IL-32γ is not categorized in any known cytokine family and the molecular character is seldom known (*1*). Nevertheless, the molecule induces potent proinflammatory cytokine like TNFα and IL-8 in human THP-1 cells and murine Raw 264.7 cells via the undiscovered receptor, and activates classic cytokine signaling pathways involving NF-κB and p38-MAPK, which proves the molecule a cytokine (*2*). The pro-protein of human IL-32 contains 234 amino acids (aa) that is composed of a 30 aa signal peptide and 204 aa mature protein with 3 potential myristoylation sites and a potential N-linked glycosylation site. IL-32γ is the representative protein among IL-32 isoforms (*1*, *2*). There are 5 potential splice variants in IL-32 isoforms (*3*). IL-32α is missing two splicing variant regions known in IL-32γ (aa 19-64 and aa 154-210) while IL-32β lacks aa 19-64 and IL-32δ, aa 19-64. IL-32ε and IL-32ζ are novel isoforms that have not been fully characterized. Human IL-32γ is active in mouse cells even though no rodent orthologs have been reported (*2*). The receptor of IL-32 has not been found, yet it was proved that neutrophil proteinase 3 (PR3) is bound to isoform IL-32α by ligand affinity chromatography (*4*, *5*). IL-32 is involved in activation induced cell death in T cells and differentiation of monocytes to macrophages in unknown lineages (*3*, *6*). Furthermore IL-32 is highly expressed in numerous pathologic tissues including the synovial tissue in rheumatoid arthritis (*4*, *7*) and epithelial cells of human colons in Crohn's disease (*4*). siRNA method proved that decrement of endogenous IL-32 in primary human blood monocytes leads the down regulation of IFNγ, TNFα and IL-6, which means IL-32 is upstream in monocytic cytokine cascade (*8*). Increased levels of IL-32 may play a protective role in human immunodeficiency virus (HIV) infection by suppressing the viral replication (*9*). Moreover, mycobacteria species including M. tuberculosis potentiates the production of IL-32 from human monocytes and macrophages (*10*).

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3. C. Goda *et al.*, *Int Immunol* **18**, 233 (Feb, 2006).
4. C. A. Dinarello, S. H. Kim, *Ann Rheum Dis* **65 Suppl 3**, iii61 (Nov, 2006).
5. D. Novick *et al.*, *Proc Natl Acad Sci U S A* **103**, 3316 (Feb 28, 2006).
6. M. G. Netea *et al.*, *Proc Natl Acad Sci U S A* **105**, 3515 (Mar 4, 2008).
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8. M. F. Nold *et al.*, *J Immunol* **181**, 557 (Jul 1, 2008).
9. S. T. Rasool *et al.*, *Immunol Lett* **117**, 161 (May 15, 2008).
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