REFERENCES

1.Muh, H. et al. (2000) Biochem. Biophys. Res. Commun. 267:960.

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3.Calderara, S. et al. (2001) Virology 279:22.

DESCRIPTION

Mouse IL-18 Immunoassay

Catalog Number:SEKM-0019

For the quantitative determination of mouse interleukin-18 (IL-18) concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IL-18 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	92	94
	Range(%)	86-103	90-103
1:4	Average% of Expected	98	97
	Range(%)	84-106	93-112

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 23.44 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant Mouse IL-18. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of IL-18 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IL-18 in two matrices

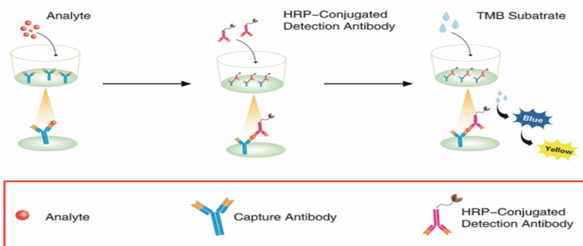
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	91	85-101
Cell culture supernatants	103	96-108

BACKGROUND

Interleukin 18 (IL-18), also known as interferon-gamma-inducing factor (IGIF) and IL-1γ, is a cytokine which shares biologic activities with IL-12 and structural similarities with the IL-1 family of proteins. IL-18 was originally cloned from liver cells and has since been shown to be expressed by monocyte/macrophages, osteoblasts and keratinocytes. Caspase-1 (IL-1 beta-converting enzyme) has been implicated in the physiological processing of pro-IL-18 to IL-18. Similarly to IL-12, human IL-18 has been shown to enhance NK cell activity in PBMC cultures. Human IL-18 has also been found to induce the production IFN-γ and GM-CSF while inhibiting the production of IL-10 by PBMC. On enriched human T cells, human IL-18 can enhance Th1 cytokine production and stimulate cell proliferation via an IL-2-dependent pathway. In the mouse system, IL-18 has been shown to be a costimulatory factor for the activation of Th1, but not Th2, cells. IL-18 was found to selectively enhance the FasL-mediated cytotoxicity of Th1, but not Th0 or Th2, cells. IL-18 has also been shown to induce activated B cells to produce IFN-γ that inhibits IgE production.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-18 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-18 present is captured by the coated antibody after incubation. Following extensive washing, a HRP-conjugated antibody specific for IL-18 is added to detect the captured IL-18 protein in sample. The wells are then washed to remove unbound HRP-labled antibody and Tetramethyl-benzidine (TMB) reagent is added. Incubated at room temperature, only those wells that contain IL-18, HRP-labled antibody will appear blue in color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450nm. The OD value is proportional to the concentration of Mouse IL-18.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

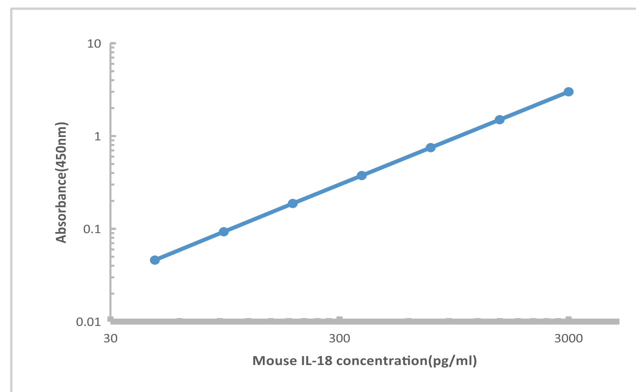
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IL-18 ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.040	0.044	-
46.88	0.172	0.192	0.182	0.138
93.75	0.250	0.279	0.265	0.221
187.5	0.372	0.415	0.393	0.350
375	0.591	0.659	0.625	0.581
750	0.960	1.070	1.015	0.971
1500	1.563	1.742	1.652	1.608
3000	2.546	2.838	2.692	2.648



Representative standard curve for IL-18 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed,wash the plate 3 times before the assay.

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

Aspirate and wash 4 times

Add 100μl working solution of HRP-Conjugate anti-Mouse IL-18 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 3-15 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the IL-18 concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized, 3000pg/ml upon reconstitution	2 vials	Store at 2-8°C**for six months
HRP-Congugated Antibody（100 ×） - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard/sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
HRP Congugated Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

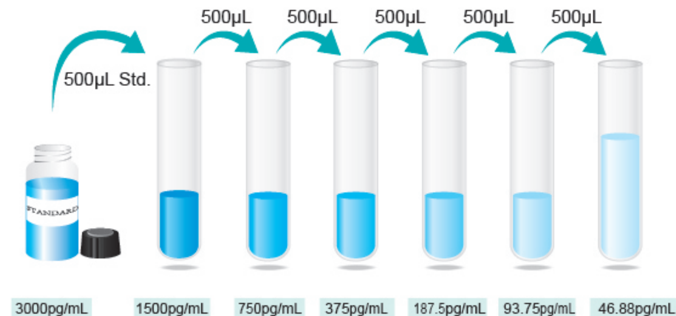
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma -Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning -** Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer -** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Sample -** Reconstitute the Standard with 1 mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 3000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500μL of Standard/Sample Diluent into 1500pg/ml tube and the remaining tubes. Use the stock solution of 3000pg/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 3000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).
***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of HRP-Congugated Antibody(100×):Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100uL of HRP-Congugated Antibody to 10mL of HRP-Congugated Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months.**
DO NOT FREEZE



Preparation of IL–18 standard dilutions