Rat IL-18 ELISA Kit

Technical Manual No. 0345 **Version 07072008**

I. INTRODUCTION

GenScript's Rat IL-18 ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. A polyclonal antibody specific for rat IL-18 has been precoated onto a 96-well plate. Standards and samples are added into the wells and any IL-18 present is bound by the immobilized antibody. After washing away any unbound substances with PBS or TBS buffer, a biotinylated detection polyclonal antibody specific for rat IL-18 is added to the wells. Following a wash to remove any unbound biotinylated antibody, avidin-biotin-peroxidase Complex substrate, or HRP substrate, TMB is used to visualize enzymatic coloration reaction. TMB is catalyzed by HRP to turn blue that changes to yellow after coming into contact with the acidic stop solution. The density of the yellow pigment is proportional to the quantity of rat IL-18 captured of samples in plate.

II. KIT CONTENTS

Kit Components	96-Well
Lyophilized recombinant rat IL-18 standard	2 Tubes (10 ng/tube)
96-well plate precoated with anti-rat IL-18 antibody	1 (12 strips of 8 wells)
Sample diluent buffer	30 ml
Biotinylated anti-rat IL-18 antibody	130 µl, dilution 1:100
Antibody diluent buffer	12 ml
Avidin-biotin-peroxidase complex (ABC)	130 µl, dilution 1:100
ABC diluent buffer	12 ml
TMB color developing agent	10 ml
TMB stop solution	10 ml
Protocol	1

III. APPLICATIONS

This kit is designed for quantitive detection of rat IL-18 in sera, plasma, body fluids, tissue lysates, and cell culture supernates.

IV. KEY FEATURES

- **Easy to perform:** The 96-well plate precoated with antibody has simple and rapid procedure to perform.
- **High sensitivity:** The kit can assay the concentration of IL-18 more than 1 pg/ml.
- **Large detection range:** The kit can detect IL-18 from 15.6 pg/ml to 1,000 pg/ml.
- **Super specificity:** There is no detectable cross-reactivity with any other cytokine.
- **Reproducible results:** The kit produces highly reproducible results.

V. STORAGE

This kit remains stable for at least eight months if stored at -20°C and for at least four months if stored at 4°C. Store any kit meant for frequent use at 4°C. Avoid multiple thawing and freezing cycles.

VI. RAT IL-18 ELISA KIT PROTOCOL

Note:

1. Before using the kit, spin tubes to bring all components to the bottom.
2. Duplicate well assays are recommended for both standard and sample test.
3. Keep the 96-well plate wet. Otherwise the activity of components in the plate will be lost.
4. Because temperature can affect results, we recommend heating the diluted ABC and TMB solution to 37°C for 30 minutes before use.

Items Needed But Not Provided In The Kit:

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and tips: A multichannel pipette is necessary for considerable samples.
4. Clean Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

   Preparation 0.01 M TBS: Add 1.2 g Tris, 8.5 g NaCl, and 450 µl of purified acetic acid (or 700 µl of concentrated hydrochloric acid) into distilled water. Adjust pH to 7.2-7.6 and the total volume to 1 L.

   Preparation 0.01 M PBS: Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4, and 0.2 g NaH_2PO_4 into distilled water. Adjust pH to 7.2-7.6 and the total volume to 1 L.

Preparation Before Experiment

- **Methods for Plate Washing**

  By hand: Absorb or throw the liquid in the plate. Do not touch well wall. Pat the plate downwards several times on fresh towels. Infuse PBS or TBS buffer (at least 0.3 ml) into wells and incubate 1-2 minutes. Repeat this process several times if necessary.
  By machine: Use the automated plate washer proficiently like formal experiments.

- **Sample Preparation and Storage**

  Cell culture supernates, tissue lysates, and body fluids
  Remove particulates by centrifugation and assay immediately, or aliquot and store samples at -20°C.

  Serum
  Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature. Then collect samples after centrifuging at approximately 1000X g for 15 minutes. Remove serum and assay immediately, or aliquot and store samples at -20°C.

Plasma
Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000X g within 30 minutes of collection. Assay immediately, or aliquot and store samples at -20°C. Avoid samples multiple freeze-thaw cycles.

- **Principles for Sample Dilution**

In order to the optimal detection range for this kit, estimate the concentration of IL-18 in the sample. It is recommended that different dilution methods for each sample be taken. The data below is for reference only.

High concentration
The concentration of purpose proteins is in the range of 10-100 ng/ml, and the working dilution ratio is 1:100. Add 3 µl of sample into 297 µl of sample diluent buffer.

Middle concentration
The concentration of purpose proteins is in the range of 1-10 ng/ml, and the working dilution is 1:10. Add 25 µl of samples into 225 µl of sample diluent buffer.

Low concentration
The concentration of purpose proteins is in the range of 15.6-1,000 pg/ml, and the working dilution ratio is 1:2. Add 100 µl of sample into 100 µl of sample diluent buffer.

Especially Low concentration
The concentration of purpose proteins is ≤15.6 pg/ml. Dilute to 1:2 or, for very dilute samples, not at all.

- **Reagent Preparation and Storage**

A. Prepare rat IL-18 standard: IL-18 standard solution is prepared within two hours of use. The kit provides two tubes of IL-18 standard (10ng per tube).
   i. Prepare 10,000 pg/ml of rat IL-18 stock standard: Add 1 ml sample diluent buffer into one tube containing 10 ng of IL-18 standard. Keep it at room temperature for 10 minutes and mix completely. This will produce IL-18 standard stock solution at a concentration of 10,000 pg/ml.
   ii. Preparation serial rat IL-18 standards with a concentration of 15.6 pg/ml to 1000 pg/ml: Label Eppendorf tubes from one to seven. Aliquot 0.9 ml and 0.3 ml of sample diluent buffer into the first tube and the other tubes, respectively. Add 0.1 ml of 10,000 pg/ml of stock standard solution into the first tube and mix thoroughly. And then, take 0.3 ml of IL-18 solution from the first one to the second one and mix. Use the same way to make later serial dilution standards.

Notice: The stock standard (10,000 pg/ml) should be used within 12 hours at 4°C. It can also be used as much as two days later if it is stored at -20°C. And avoid multiple freeze-thaw cycles.

B. Prepare biotinylated anti-rat IL-18 antibody working solution.
Dilute biotinylated anti-rat IL-18 antibody with antibody diluent buffer at a ratio of 1:99 and mix thoroughly. 0.1 ml of the resultant mixture is required per well. Determine total volume of the precasted mixture according with the number of assays. The solution should be prepared within two hours before use.

C. Prepare avidin-biotin-peroxidase complex (ABC) working solution.
Dilute avidin-biotin-peroxidase complex (ABC) with ABC dilution buffer at a ratio of 1:99 and mix thoroughly. 0.1 ml of the resultant mixture is required per well. Determine total volume of the precasted mixture according with the number of assays. The solution should be prepared one hour before use.

Assay Procedure

The ABC working solution and TMB color developing agent should be warmed to 37°C for 30 minutes before use. Keep diluted samples and reagents mixed completely. Standard IL-18 detection curve should be prepared for each experiment. Decide appropriate dilution fold for samples.

1. Pipette 0.1 ml of IL-18 standard prepared (1,000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/m, 31.3 pg/ml, 15.6 pg/ml) into precoated wells, respectively. 0.1 ml of sample diluent buffer serves as the control well (often labeled “well zero”). Prepare samples as described as above and add 100 µl of diluted samples directly into wells. It is recommended that each IL-18 standard and sample should be assayed in duplicate.
2. Seal plate with cover and incubate at 37°C for 90 minutes.
3. Remove cover and discard the solution in each well. Strike plate on fresh towels to make wells clean, but do not allow them to dry completely at any time.

4. Add 0.1 ml of biotinylated anti- rat IL-18 antibody working solution into each well and incubate the plate at 37°C for 60 minutes.
5. Wash plate three times with washing buffer prepared, and keep washing buffer in wells for one minute each time.
6. Add 0.1 ml of ABC working solution prepared into each well and incubate at 37°C for 30 minutes.
7. Wash plate five times with washing buffer, and keep washing buffer in wells for 1-2 minutes each time.
8. Add 90 µl of TMB color developing agent provided into each well, and incubate at 37°C for 15-20 minutes (blue color gradient can be visually observed from the first to fourth dilution well of IL-18 standard sample dilutions, the rest wells show no color difference obviously).
9. Add 0.1 ml of TMB stop solution provided into each well. The color should change to yellow immediately.
10. Read O.D. absorbance with microplate reader at 450nm within 30 minutes after adding stop solution.
11. The IL-18 standard curve can be draw using IL-18 concentration (X) vs absolute O.D. 450 value (Y). The absolute O.D. 450= O.D. 450 of IL-18 standard or sample –O.D. 450 of well zero. The IL-18 concentration of samples can be calculated from the IL-18 standard curve.

Notice: The real IL-18 concentration of sample = IL-18 concentration of sample obtained from the standard curve × sample dilution fold (N).

Conclusion

1. Add samples and standards and incubate the plate at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 minutes. Wash plate three times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 minutes. Wash plate five times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 15-20 minutes.
5. Add TMB stop solution and read.

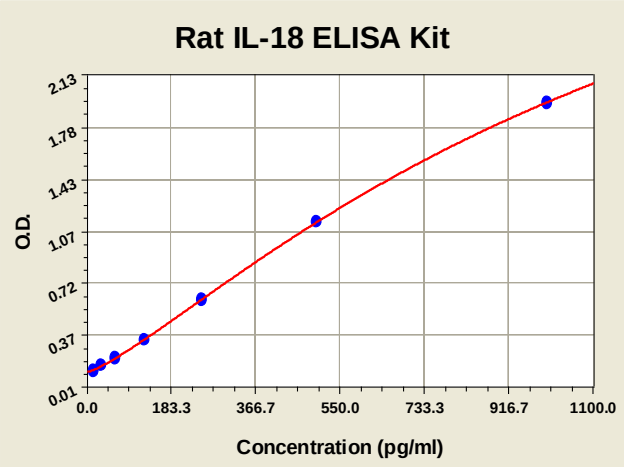
VII. EXAMPLES

Typical Data Obtained Using IL-18 Standard

(Incubate TMB with antibody at 37°C for 17 minutes.)

Concentration (pg/ml)	0	15.6	31.3	62.5	125	250	500	1,000
O.D.	0.112	0.129	0.165	0.216	0.331	0.612	1.138	1.951

Rat IL-18 ELISA Kit - 1 x 96 Well Plate Images



VIII. BACKGROUD AND REFERENCES

Background:

Interleukin (IL)-18, also called Interferon-gamma-inducing factor (IGIF), augments natural killer (NK) activity in spleen cells. The IL-18 gene encodes a precursor protein of 192 amino acids and a mature protein of 157 amino acids.[1] IL-18 is a recently discovered cytokine that modulates both T helper type 1 (Th1) and Th2 responses.[2] IL-18 is a potent proinflammatory cytokine with potential atherogenic properties. It is highly expressed in the atherosclerotic plaques compared with control normal arteries and is localized mainly in plaque macrophages.[3] The standard product used in this kit is recombinant rat IL-18, consisting of 159 amino acids with the molecular mass of 18.4 kDa.

References:

1. Okamura, H.; Tsutsui, H.; Komatsu, T.; Yutsudo, M.; Hakura, A.; Tanimoto, T.; Torigoe, K.; Okura, T.; Nukada, Y.; Rattori, K.; Akita, K.; Namba, M.; Tanabe, F.; Konishi, K.; Fukuda, S.; Kurimoto, M. Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 378: 88-91, 1995.
2. Reddy, P.; Teshima, T.; Kukuruga, M.; Ordemann, R.; Liu, C.; Lowler, K.; Ferrara, J. L. M. Interleukin-18 regulates acute graft-versus-host disease by enhancing Fas-mediated donor T cell apoptosis. *J. Exp. Med.* 194: 1433-1440, 2001.
3. Mallat, Z.; Corbaz, A.; Scoazec, A.; Besnard, S.; Leseche, G.; Chvatchko, Y.; Tedgui, A. Expression of interleukin-18 in rat atherosclerotic plaques and relation to plaque instability. *Circulation* 104: 1598-1603, 2001.

IX. ORDERING INFORMATION

Rat IL-18 ELISA Kit: Cat.No.L00390.

GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com

For Research Use Only.