

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

Quantitative test kit for Mouse IL-18

# **Mouse IL-18 ELISA Kit**

**CODE. No. 7625**

## **CONTENTS**

1. Intended Use -----	1
2. Summary and Explanation -----	1
3. Principle -----	2
4. Materials provided -----	2
5. Materials and equipment required -----	2
6. Procedure -----	3
7. Precaution -----	5
8. Storage and Stability -----	6
9. Performance Characteristics -----	6
10. References -----	8

**Before use, thoroughly read these Instructions.**

### **Intended Use**

The Mouse IL-18 ELISA Kit is based on sandwich ELISA and capable of measuring mouse IL-18.

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

### **Summary and Explanation**

Interleukin 18 (IL-18) is an 18 kDa novel cytokine which is identified as a costimulatory factor for production of interferon- $\gamma$  (IFN- $\gamma$ ) in response to toxic shock. It shares functional similarities with IL-12. IL-18 is synthesized as a precursor 24 kDa molecule without a signal peptide and must be cleaved to produce an active molecule. IL-1 converting enzyme (ICE, Caspase-1) cleaves pro-IL-18 at aspartic acid in the P1 position, producing the mature, bioactive peptide that is readily released from cells. It has been reported that IL-18 is produced by Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts, adrenal cortex cells and murine diencephalon. IFN- $\gamma$  is produced by activated T and NK cells and plays critical roles in the defense against microbial pathogens. IFN- $\gamma$  activates macrophages, enhances NK activity and B cell maturation, proliferation and Ig secretion, induces MHC class I and II antigens expression, and inhibits osteoclast activation.

IL-18 acts on T helper 1-type T (Th1) cells and in combination with IL-12 strongly induces production of IFN- $\gamma$  by these cells. Pleiotropic effects of IL-18 have also been reported, including enhancement production of IFN- $\gamma$  and GM-CSF in peripheral blood mononuclear cells, production of T helper type 1 cytokines, IL-2, GM-CSF and IFN- $\gamma$  in T cells, enhancement of Fas ligand expression by T helper type 1 cells.

The "Mouse IL-18 ELISA Kit " is the best reagent for specifically measuring mouse IL-18 with high sensitivity by ELISA.

1 ng/ml of the cytokines, Mouse IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-4, IL-5, IL-6, IL-10, IL-12, GM-CSF and Human IL-18 were measured by this ELISA. The results were all below the detection limit of 25.0 pg/ml.

## **Principle**

The Mouse IL-18 ELISA Kit measures Mouse IL-18 by sandwich ELISA. The assay uses two monoclonal antibodies against two different epitopes of Mouse IL-18.

In the wells coated with anti-Mouse IL-18 monoclonal antibody, samples to be measured or standards are incubated. After washing, a peroxidase conjugated anti-Mouse IL-18 monoclonal antibody is added into the microwell and incubated. After another washing, the peroxidase substrate is mixed with the chromogen and allowed to incubate for an additional period of time. An acid solution is then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density (O.D.) of each well is then measured at 450 nm using a microplate reader. The concentration of Mouse IL-18 is calibrated from a dose response curve based on reference standards.

## **Materials provided**

Each kit contains;

Materials	Quantity (96wells)
Microwell strips coated with anti-Mouse IL-18 antibody	8-well strip x 12 strips
Mouse IL-18 calibrator ( <b>Lyophilized</b> )	2 vials
Conjugate reagent (Peroxidase conjugate anti-mouse IL-18 monoclonal antibody) ( <b>x101</b> )	0.2 ml x 1 vial
Conjugate diluent (ready to use)	24 ml x 1 vial
Assay diluent (ready to use)	30 ml x 1 vial
Washing buffer (powder)	3 packages
Substrate reagent (TMB/H <sub>2</sub> O <sub>2</sub> ) (ready to use)	15 ml x 1 vial
Stop solution (2N H <sub>2</sub> SO <sub>4</sub> ) (ready to use) (irritant)	18 ml x 1 vial

## **Materials and equipment required**

- \* Microplate reader
- \* Plate washer or washing bottle
- \* Adjustable micropipette
- \* Multichannel micropipette
- \* 96-well polyvinyl plate
- \* Microplate holder
- \* Uncoated microwell strips (for using Auto washer)
- \* Reagent vessel
- \* Distilled water

## **Procedure**

### ◆ Preparation of Reagents

#### 1. Wash solution

The Washing buffer must be dissolved in distilled water prior to use. Just before substrate incubation, dissolved one (1) package in 1,000 ml of distilled water. Prepare only a sufficient amount of wash solution for the assay, because wash solution can not be stored.

#### 2. Conjugate solution

Peroxidase conjugated anti-mouse IL-18 monoclonal antibody must be diluted prior to use. Dilute the Peroxidase conjugated anti-mouse IL-18 monoclonal antibody 1:101 with Conjugate diluent.e.g. by adding 10 µl of the Peroxidase conjugated anti-mouse IL-18 monoclonal antibody to 1,000 µl of the Conjugate diluent.

\*Prepare only a sufficient amount of the conjugate solution for the assay because the diluted conjugate is not stable.

\*Use disposable new pipette and vessel to avoid contamination by microbes.

#### 3. Standards

Reconstitute with the volume of Assay diluent, and dilute the reconstituted calibrator as indicated in "preparation of Standards".

\*If reconstituted calibrator is needed to store, prepare appropriate aliquots and freeze them below -20°C. Avoid repeated freezing and thawing.

#### **4. Other reagents are ready-to-use.**

### ◆ Preparation of samples

#### 1. Dilution

Dilute each sample with Assay diluent.

e.g. Mouse serum

1:5 with Assay diluent, e.g. by adding 50 µl of sample to 200 µl of Assay diluent. Optimal sample dilution may vary between different specimens. Appropriate sample dilution should be established by each investigator.

**Since Mouse IL-18 calibrator contains FCS, any sample which does not contain serum component (such as FCS) may result in different reactivity than the sample that contains the serum component (e.g. mouse serum, mouse plasma and mouse derived cell culture supernatant containing FCS). Thus, this kit IS NOT SUITABLE FOR MEASURING THE SAMPLE WHICH DOES NOT CONTAIN ANY SERUM COMPONENT.**

#### 2. Storage

Fresh samples should be used. Aliquot each sample into new plastic tube and store below -20°C if necessary. Avoid repeated freezing and thawing.

◆ Assay procedure

Duplicate assay will be recommended.

STEP 1. (Sample incubation)

1) Add 150 µl of prepared samples and standards to 96-well polyvinyl plate as the same order of assay run. Then, transfer 100 µl of each sample to Mouse IL-18 antibody coated microwells simultaneously using multichannel pipette.

\*Reaction starts on pipetting to Mouse IL-18 antibody coated microwell. Pipetting should be completed as quickly as possible.

2) Incubate for 60 minutes at room temperature (20 - 25°C) .

STEP 2. (Washing)

Aspirate or discard the well contents. Fill the wells with Wash solution and then completely aspirate or discard the contents. Wash the well 4 times with wash solution using washing bottle. When an autowasher is used, wash 4 times.

\*Each laboratory is recommended to confirm its own appropriate washing times and set-up.

\*Washing buffer should be used at room temperature (20 - 25°C) .

STEP 3. (Conjugate incubation)

1) Pour conjugate solution into the the vessel. After removing wash solution remained completely, pipette 100 µl of conjugate solution to each well with multichannel pipette.

**\*To avoid drying of the microwells, the conjugate solution must be dispensed into the microwells immediately upon removal of the wash solution.**

2) Incubate for 60 minutes at room temperature (20 - 25°C) .

STEP 4. (Washing)

Wash the microplate again following the STEP 2 procedure.

STEP 5 (Substrate incubation)

1) Pour Substrate reagent into the vessel. Add 100 µl of Substrate reagent to each well.

\*Substrate reagent should be used at room temperature (20 - 25°C) .

\*This vessel should be different from the one which was used for pouring conjugate solution.

\*Use disposable new pipette and vessel, as Substrate reagent is easily oxidized by metal ions and may be contaminated by microbes.

\*If Substrate reagent is poured into the vessel from the bottle, do not return to the bottle.

**\*To avoid drying of the microwells, the Substrate reagent must be dispensed into the microwells immediately upon removal of the wash solution.**

2) Incubate for 30 minutes at room temperature (20 - 25°C).

**STEP 6. (Stopping reaction)**

Pour Stop solution into the vessel. Pipette 100  $\mu$ l of Stop solution to each well with multichannel pipette.

◆ Reading

Read the absorbance of each well at 450 nm. If a dual wavelength plate reader is available, set the test wave length at 450 nm and the reference at 620 nm.

\*Reading should be done within 30 minutes after stopping reaction.

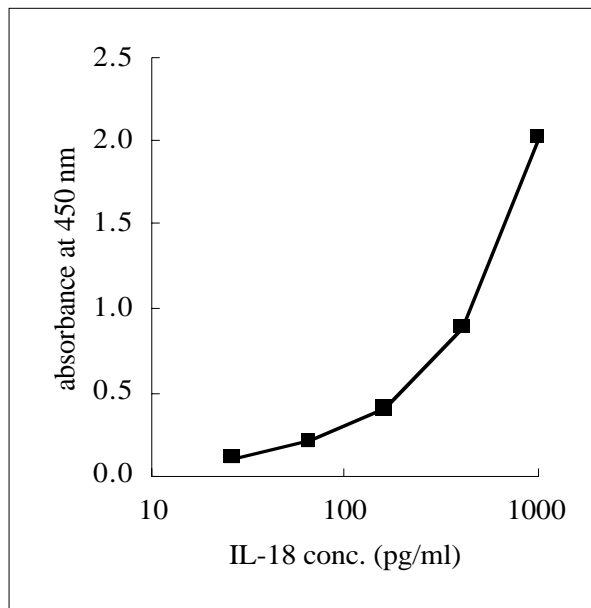
◆ Calculation of results

Calculate the mean absorbance value of each standard. Plot on the semi-log graph paper and construct a standard curve (Absorbance on the vertical axis, concentration (in pg/ml) on the horizontal axis).

Report the IL-18 concentration of samples by multiplying the value read from the standard curve by dilution factor (e.g. Mouse sera; x 5).

\*If absorbance of sample exceeds the value of the 1,000 pg/ml standard, dilute the sample and measure again.

◆ Example of Standard curve



**Precaution**

1. Allow all the components to come to room temperature (20 - 25°C) before use.
2. All microwell strips which are not immediately required should be returned to the ziplock pouch, which must be carefully resealed to avoid moisture absorption.
3. Fresh samples should be used. Aliquot each sample and store below -20°C if necessary.

Avoid repeated freezing and thawing. Never store the samples at 4°C, as samples are affected

by storage at this temperature.

4. Assay diluent contains sodium azide (0.1%) as preservative. Azide may react with copper or lead in plumbing systems to form explosive metal azide. Therefore, always flush drains with plenty of water when disposing of materials containing azide.
5. Stop solution is 2N sulfuric acid. As it is a corrosive product, protect eyes and skin and handle with care.
6. **This kit is intended for research use only. Not for use in diagnostic procedure.**

### **Storage and Stability**

All kit components must be stored at 2-8°C. All reagents are stable for 6 months after manufacturing when stored at the conditions indicated.

### **Performance Characteristics**

#### ◆ Sensitivity

The sensitivity of the assay is 25.0 pg/ml.

The minimum detection limit estimated by serial dilution was 25.0 pg/ml since the mean +2 S.D. of the 12.5 pg/ml was lower than the mean -2 S.D. of the 25.0 pg/ml.

#### ◆ Reproducibility

##### 1. Intra-assay

Intra-assay reproducibility was determined by assaying the sera 8 times.

IL-18 concentrations of the serum samples were calculated as described in calculation of results in assay procedure.

Sample	serum 1	serum 2	serum 3	serum 4	serum 5	serum 6
Number of replicates	8	8	8	8	8	8
Mean (pg/ml)	3554.4	3285.9	2791.7	2212.8	992.3	625.3
C.V. (%)	2.5	2.0	5.8	5.3	3.1	3.8

##### 2. Inter-assay

Inter-assay reproducibility was determined by 5 independent assays of the sera.

IL-18 concentrations of the serum samples were calculated as described in calculation of results in assay procedure.

Sample	serum 1	serum 2	serum 3	serum 4	serum 5
Number of determinations	5	5	5	5	5
Mean (pg/ml)*	3500.2	3241.7	2557.9	2066.8	842.6
C.V. (%)	7.4	4.4	3.8	5.4	8.5

\*From duplicate of each serum sample in five (5) separate assays.

## ◆ Recovery test

Recombinant mouse IL-18 was added to sample at different concentrations.

IL-18 concentrations of the serum samples were calculated as described in calculation of results in assay procedure.

## Serum 1

(A) Additional rmIL-18* (pg/ml)	IL-18 concentration observed (pg/ml)	(B) Recovery (pg/ml)	(B/A) Recovery (%)
0.0	956.0	-	-
536.0	1497.3	541.3	101.0
960.5	1873.3	917.3	95.5

\*rmIL-18 is abbreviation of recombinant mouse IL-18.

## Serum 2

(A) Additional rmIL-18* (pg/ml)	IL-18 concentration observed (pg/ml)	(B) Recovery (pg/ml)	(B/A) Recovery (%)
0.0	742.0	-	-
536.0	1279.0	537.0	100.2
960.5	1594.0	852.0	88.7

\*rmIL-18 is abbreviation of recombinant mouse IL-18.

## Serum 3

(A) Additional rmIL-18* (pg/ml)	IL-18 concentration observed (pg/ml)	(B) Recovery (pg/ml)	(B/A) Recovery (%)
0.0	1397.3	-	-
536.0	1921.8	524.5	97.9
960.5	2213.0	815.8	84.9

\*rmIL-18 is abbreviation of recombinant mouse IL-18.

## Serum 4

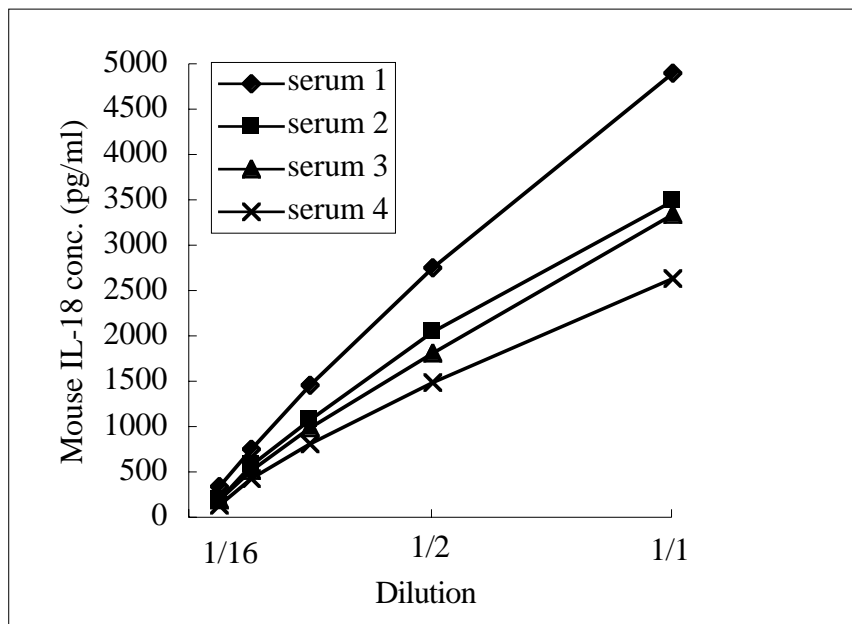
(A) Additional rmIL-18* (pg/ml)	IL-18 concentration observed (pg/ml)	(B) Recovery (pg/ml)	(B/A) Recovery (%)
0.0	340.8	-	-
536.0	884.5	543.8	101.4
960.5	1278.8	938.0	97.7

\*rmIL-18 is abbreviation of recombinant mouse IL-18.

◆ Dilution test

Samples were diluted with Assay diluent.

IL-18 concentrations of the serum samples were calculated as described in calculation of results in assay procedure.



**REFERENCE:**

Okamura H., et al. *Nature* **378**, 88-91 (1995)  
 Ushio S., et al. *J. Immunol.* **156**, 4274-4279 (1996)  
 Micallef M., et al. *Eur. J. Immunol.* **26**, 1647-1651 (1996)  
 Tao D, et al. *Cell Immunol.* **173**, 230-235 (1998)  
 M. Taniguchi, et al. *J. Immunol. Methods* **206**, 107-113 (1997)