

PRODUCT INFORMATION & MANUAL

Human IL-18 Instant ELISA

BMS267INST

Enzyme-linked immunosorbent assay for
quantitative detection of human IL-18.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests



*Human IL-18
Instant ELISA*

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1 Intended Use

The human IL-18 Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-18. **The human IL-18 Instant ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

Interleukin (IL) -18 is a newly discovered Cytokine, structurally similar to IL-1, with profound effects on T-cell activation.

Formerly called interferon (IFN) gamma inducing factor (IGIF), IL-18 is a novel cytokine that plays an important role in the T-cell-helper type 1 (Th1) response, primarily by its ability to induce IFN gamma production in T cells and natural killer (NK) cells.

In terms of structure, IL-18 and IL-1 beta share primary amino acid sequences of the so-called „signature sequence“ motif and are similarly folded as all beta pleated sheet molecules. Also similar to IL-1 beta, IL-18 is synthesized as a biologically inactive precursor molecule lacking a signal peptide which requires cleavage into an active, mature molecule by the intracellular cysteine protease called IL-1 beta-converting enzyme (ICE, caspase-1). Therefore inhibitors of ICE activity may limit the biologic activity of IL-18 and may be useful as Th1 immunosuppressive agents.

The activity of the mature IL-18 is closely related to that of IL-1.

IL-18 induces gene expression and synthesis of tumor necrosis factor (TNF), IL-1, Fas Ligand, and several chemokines.

IL-18 induces the differentiation of Th1 or Th2 cells depending upon cytokine milieu and genetic background.

The activity of IL-18 is via an IL-18 receptor (IL-18R) complex. This complex is made up of a binding chain termed IL-18R₁, a member of the IL-1 receptor family previously identified as the IL-1 receptor – related protein (IL-1Rrp), and a signaling chain, also a member of the IL-1R family.

The IL-18R complex recruits the IL-1R – activating kinase (IRAK) and TNF-R – associated factor –6 (TRAF-6) with subsequent activation of NF kappa B.

Thus based on primary structure, three-dimensional structure, receptor family, signal transduction pathways and biological effects, IL-18 appears to be a new member of the IL-1 family.

Similar to IL-1, IL-18 participates in both innate and acquired immunity.

Constitutive IL-18 expression is detected from many different cells, including macrophages, keratinocytes, and osteoblasts. Human keratinocytes have been found to be the major producers of IL-18 in unprocessed form.

An important role of IL-18 has been shown in clinical implications, in the role of IL-18 modulation in tumours, infections, and autoimmune and inflammatory diseases.

Elevated serum levels of IL-18 have been shown in patients with acute graft-versus-host disease after bone marrow transplantation.

Highly elevated IL-18 bioactivity with highly elevated serum IL-18 levels are measured in hematological malignancies as well as in sepsis.

Increased concentrations of plasma IL-18 are found in patients with hepatic dysfunction after hepatectomy and in inflammatory liver diseases. Elevations of IL-18 levels accompany autoimmune disorders like systemic lupus erythematosus and rheumatoid arthritis.

Significant levels of circulating IL-18 are found in HIV-1 infection, and other infections, in inflammatory CNS diseases, in Crohn's disease and in pregnancy during labour and complicated pregnancies.

IL-18 is regarded as a novel player in tumor immunotherapy.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

An anti-human IL-18 coating antibody is adsorbed onto microwells. Human IL-18 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human IL-18 antibody binds to human IL-18 captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human IL-18.

Following incubation unbound biotin conjugated antihuman IL-18 and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of soluble human IL-18 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-18 standard dilutions and human IL-18 sample concentration determined.

Figure 1

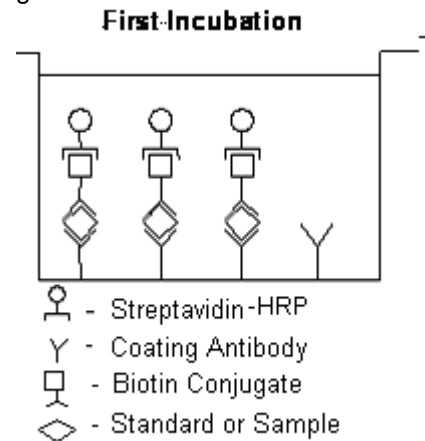


Figure 2

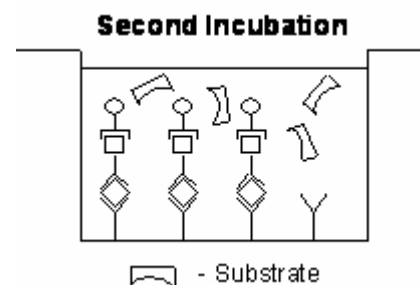
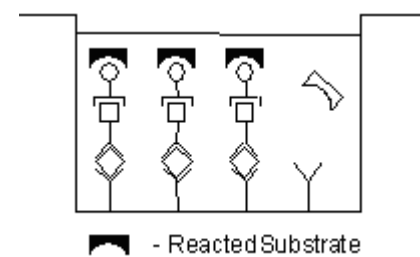


Figure 3



4 Reagents Provided

- 1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human IL-18, **HRP-Conjugate** (human IL-18 monoclonal antibody) and Sample Diluent, lyophilized
- 2 aluminium pouches with a human IL-18 **Standard curve** (coloured)
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (phosphate-buffered saline with 1% Tween 20)
- 1 vial (12 ml) **Sample Diluent** (Use when an external predilution of the samples is needed)
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant, serum, plasma (EDTA, citrate, heparin) and urine were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive human IL-18. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1000 μ l adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 μ l and 500 μ l) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

Buffer concentrate should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

10 Test Protocol

- Use plate immediately after removal from -20°C!
 - Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!
 - Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.
 - Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.
 - Allow the washing buffer to sit in the wells for a few seconds before aspiration.
 - Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.
- a. Determine the number of microwell strips required to test the desired number of samples plus microwell strips for blanks and standards (coloured). Each sample, standard and blank should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).
 - b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).
 - c. Add 100 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (5000 pg/ml)	Standard 1 (5000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (2500 pg/ml)	Standard 2 (2500 pg/ml)	Sample 2	Sample 2
C	Standard 3 (1250 pg/ml)	Standard 3 (1250 pg/ml)	Sample 3	Sample 3
D	Standard 4 (625 pg/ml)	Standard 4 (625 pg/ml)	Sample 4	Sample 4
E	Standard 5 (313 pg/ml)	Standard 5 (313 pg/ml)	Sample 5	Sample 5
F	Standard 6 (156 pg/ml)	Standard 6 (156 pg/ml)	Sample 6	Sample 6
G	Standard 7 (78 pg/ml)	Standard 7 (78 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- d. Add 50 µl of each **sample**, in duplicate, to the **designated wells** and mix the contents.
- e. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours, if available on a microplate shaker at 400 rpm.
- f. Remove adhesive film and empty wells. **Wash** the microwell strips 6 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

- g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

- i. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

- j. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human IL-18 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-18 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human IL-18 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-18 concentration.
- ***Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human IL-18 levels. Such samples require further external predilution according to expected human IL-18 values with Sample Diluent in order to precisely quantitate the actual human IL-18 level.**
- It is suggested that each testing facility establishes a control sample of known human IL-18 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 μ l to the final volume per well. These 100 μ l are composed of 50 μ l of Sample Diluent plus 50 μ l of the sample. This is a 1:2 dilution.

The remaining 50 μ l to give 150 μ l are due to the addition of 50 μ l conjugate to all wells.

50 μ l Sample Diluent and 50 μ l conjugate results in 100 μ l reconstitution volume, addition of 50 μ l sample (50 μ l + 50 μ l sample= 1:2 dilution)

Figure 4

Representative standard curve for human IL-18 Instant ELISA. Human IL-18 was diluted in serial 2-fold steps in Sample Diluent. Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

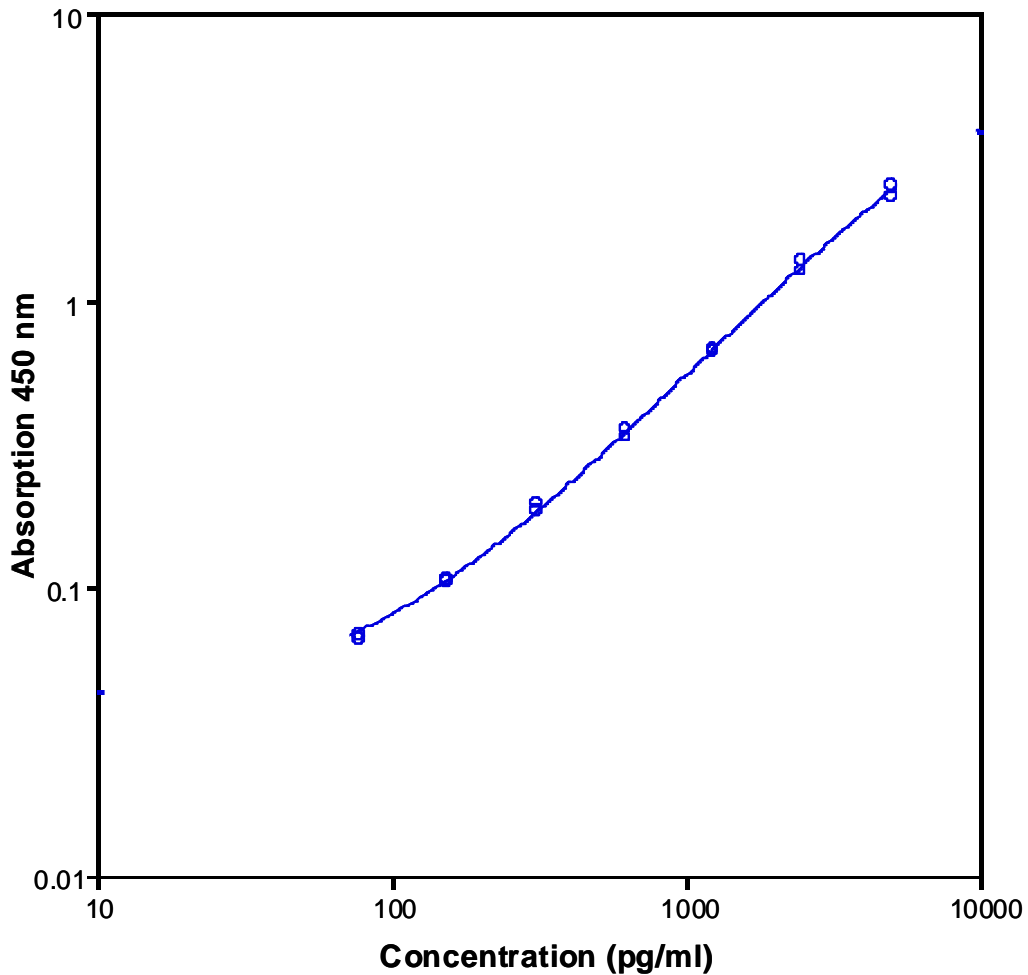


Table 2

Typical data using the human IL-18 INSTANT ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human IL-18 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	5000	2.307 2.506	2.407	5.8
2	2500	1.264 1.374	1.319	5.9
3	1250	0.671 0.679	0.675	0.8
4	625	0.332 0.356	0.344	4.9
5	313	0.197 0.186	0.192	4.1
6	156	0.108 0.105	0.107	2.0
7	78	0.068 0.066	0.067	2.1
Blank	0	0.028 0.023	0.026	9.8

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human IL-18 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 9.2 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human IL-18. 2 standard curves were run on each plate. Data below show the mean human IL-18 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.5%.

Table 3

The mean human IL-18 concentration and the coefficient of variation for each sample.

Sample	Experiment	Human IL-18 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	5717	7
	2	6478	10
	3	7286	5
2	1	2918	5
	2	3414	9
	3	3739	3
3	1	1588	4
	2	1959	6
	3	1896	9
4	1	847	10
	2	1067	6
	3	1027	5
5	1	3693	3
	2	3499	6
	3	3990	4
6	1	1851	5
	2	1769	8
	3	1835	9
7	1	848	7
	2	1052	10
	3	829	6

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human IL-18. 2 standard curves were run on each plate. Data below show the mean human IL-18 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 8.1%.

Table 4

The mean human IL-18 concentration and the coefficient of variation of each sample

Sample	Mean Human IL-18 Concentration (pg/ml)	Coefficient of Variation (%)
1	6494	9.9
2	3357	10.1
3	1814	8.9
4	980	9.7
5	3727	5.4
6	1818	2.0
7	909	11.1

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human IL-18 into serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments.

Average recovery ranged from 78% to 100% with an overall mean recovery of 83%.

13.4 Dilution Parallelism

4 serum samples with different levels of human IL-18 were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 88% and 102% with an overall recovery of 93%.

Table 5 shows detailed recovery data of 4 serum samples.

Table 5

Sample	Dilution	Expected Human IL-18 Concentration (pg/ml)	Observed Human IL-18 Concentration (pg/ml)	Recovery of Expected Human IL-18 Concentration (%)
1	1:2	--	5610.7	--
	1:4	2805.4	2726.6	97.2
	1:8	1363.3	1193.8	87.6
2	1:2	--	3184.0	--
	1:4	1592.0	1416.6	89.0
	1:8	708.3	666.8	94.1
3	1:2	--	5683.8	--
	1:4	2841.9	2643.1	93.0
	1:8	1321.6	1189.9	90.0
4	1:2	--	3538.4	--
	1:4	1769.2	1809.3	102.3
	1:8	904.7	851.3	94.1

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human IL-18 levels determined.

There was no significant loss of human IL-18 immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IL-18 level determined after 24 h. There was no significant loss of human IL-18 immunoreactivity detected during storage at -20°C, 2-8°C and room temperature.

A significant loss of human IL-18 immunoreactivity (20%) was detected during storage at 37°C.

13.6 Comparison of Serum and Plasma

Serum, urine as well as EDTA, citrate, and heparin plasma obtained from 8 individuals at the same time point were evaluated. Human IL-18 concentrations were not significantly different and therefore all these body fluids are suitable for the assay. It is nevertheless highly recommended to assure the uniformity of blood preparations.

13.7 Specificity

To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no crossreactivity observed, notably not with IL-18 precursor protein (pro-IL-18).

13.8 Expected Values

A panel of 8 sera samples from randomly selected apparently healthy donors (males and females) was tested for human IL-18.

The detected human IL-18 levels ranged between 55 and 280 pg/ml with a mean level of 130 pg/ml.

14 Ordering Information

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15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 100 µl distilled water to sample wells.
- Add 50 µl sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) if available on a microplate shaker at 400 rpm.
- Empty and wash microwell strips 6 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2)