Human IL-18 ELISA Instructions

Cat: EH0053

Content

	CAT	Volume
❶CP (Coated Plate)	EH0053CP	96 well
❷S1 (Standard)	EH0053S1	2 vial
❸SD (Sample Diluent)	ESD01	15ml/bottle
❹DA (Detect Antibody)	EH0053DA	6 ml/bottle
❺SH (Streptavidin-HRP)	ESH02	12 ml/bottle
❻AB (Assay Buffer 1×)	EAB01	12 ml/bottle
❼TS (TMB Substrate)	ETS01	12 ml/bottle
❽SS (Stop Solution)	ESS01	12 ml/bottle
❾WB (Wash Buffer 10×)	EWB01	50 ml/bottle
❿SF (Sealer Film)	ESF01	6 pieces

■ **NOTE:** After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

Sample Dilution

Samples such as serum、plasma require at least a 20-fold dilution into Sample Diluent. A suggested 20-fold dilution is 10 μl of sample + 190 μl of Sample Diluent.

REAGENT PREPARATION

■ **Washing Buffer (1×) Preparation**
Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

■ **Standard Curve Preparation:**
Reconstitute Human IL-18 Standard by addition of distilled water as S1. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 5000 pg/ml).

Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

Pipette 200 μl of Sample Diluent into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. Sample Diluent serves as the zero standard (0 pg/ml).

| 5000 pg/ml | 1666.67 pg/ml | 555.56 pg/ml | 185.19 pg/ml | 61.73 pg/ml | 20.58 pg/ml | 6.86 pg/ml |
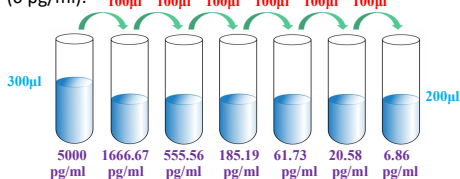
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

❶ Prepare all reagents and working standards as directed in the previous sections.

❷ Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

❸ Add 50 μl of **AB** (Assay Buffer) to each well.

❹ Add 50 μl or 10 μl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

❺ Add 50 μl of **DA** (Detect Antibody) to each well.

❻ Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 1 hour on a microplate **shaker** set at 500 rpm.

❼ Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 μl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.

❽ Add 100 μl of **SH** (Streptavidin-HRP) to each well.

❾ Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate **shaker** set at 500 rpm.
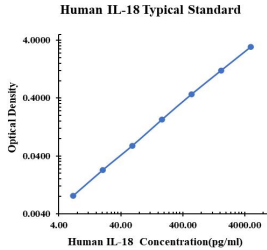
❿ Repeat aspiration/**wash** as in step 7.

⓫ Add 100 μl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

⓬ Add 100 μl of **SS** (Stop Solution) to each well.

⓭ Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA

Human IL-18 Typical Standard

pg/ml	O.D.		Average	Corrected
0.00	0.0206	0.0201	0.0204	
6.86	0.0285	0.0286	0.0286	0.0286
20.58	0.0427	0.0437	0.0432	0.0432
61.73	0.0799	0.0812	0.0806	0.0806
185.19	0.1873	0.1930	0.1902	0.1902
555.56	0.4829	0.4890	0.4860	0.4860
1666.67	1.2150	1.2240	1.2195	1.2195
5000.00	3.0220	3.1240	3.0730	3.0730

SENSITIVITY

The minimum detectable dose (MDD) of human IL-18 is typically less than 0.46 pg/ml (50 µl of sample volume) or 2.13 pg/ml (10 µl of sample volume).

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

■ **Intra-assay Precision (Precision within an assay)** Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

■ **Inter-assay Precision (Precision between assays)**

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average （pg/ml）	1439.9	447.6	84.6	1253.3	395.7	75.1
Standard Deviation	34.8	13.9	4.1	105.2	22.9	5.2
Coefficient of Variation （%）	2.4	3.1	4.8	8.7	6.6	6.1

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human IL-18 into health human serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 97% to 130% with an overall mean recovery of 109%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of IL-18 in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 100% to 118% with an overall mean recovery of 110%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of human IL-18 in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Serum	30	2.04-14.77	100	9.97

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.