

## Human IL-12 p40 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human IL-12 p40 concentrations in cell culture supernatants.

### General Information

Catalogue Number	KE00018
Product Name	Human IL-12 p40 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	3593
SwissProt	P29460

### Kit Components & Storage

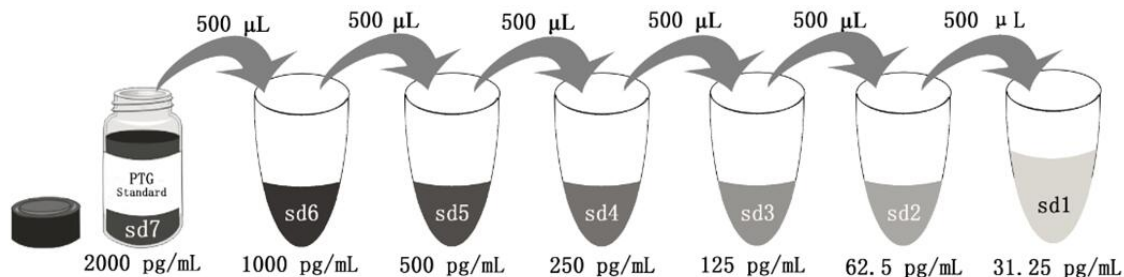
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µ L/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 1-ac - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1-ac is for protein standard and samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

\*Add 2 mL Sample Diluent PT 1-ac in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ac	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00018 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL12 p40 ELISA kit is to be used to detect and quantify protein levels of endogenous IL12 p40. The assay recognizes human IL12 p40. An antibody specific for IL12 p40 has been pre-coated onto the microwells. The IL12 p40 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IL12 p40 is added to detect the captured IL12 p40 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethylbenzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## Background

Interleukin-12 (IL-12), is a cytokine that is secreted by activated phagocytes and dendritic cells and that induces interferon- $\gamma$  production by natural-killer and T lymphocytes. IL-12 is a 75 kDa heterodimer composed of a 35 kDa subunit (IL-12A p35) and a 40 kDa subunit (IL-12B p40) that is secreted by a wide variety of antigen presenting cells (APCs), including phagocytes, B cells and Langerhans cells. IL-12 p40 has been found to be important for sustaining a sufficient number of memory/effector Th1 cells to mediate long-term protection to an intracellular pathogen. Overexpression of IL-12 p40 was observed in the central nervous system of patients with multiple sclerosis (MS), suggesting a role of this cytokine in the pathogenesis of the disease. The promoter polymorphism of IL-12 p40 has been reported to be associated with the severity of atopic and non-atopic asthma in children.

## Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is

suggested according to the individual samples.

## Safety Notes

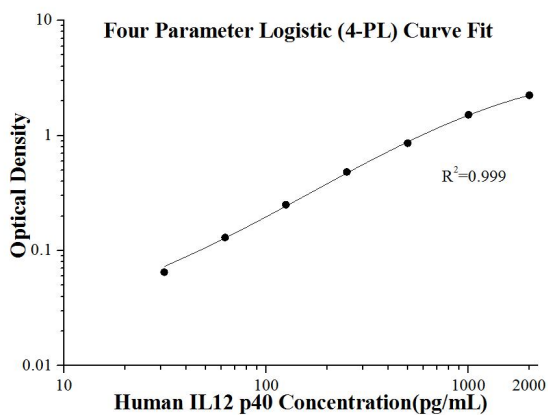
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

## Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.068 0.067	0.0675	—
31.25	0.134 0.131	0.1325	0.065
62.5	0.199 0.196	0.1975	0.13
125	0.331 0.305	0.318	0.2505
250	0.562 0.538	0.55	0.4825
500	0.917 0.935	0.926	0.8585
1000	1.581 1.585	1.583	1.5155
2000	2.293 2.317	2.305	2.2375

## Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	2026.3	122.6	6.1	1	24	1899.2	148.5	7.8
2	20	474.7	17.9	3.8	2	24	453.3	28.0	6.2
3	20	93.2	5.5	5.9	3	24	87.7	7.8	8.9

## Recovery

The recovery of IL-12 p40 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Cell culture supernatants	1:2	91	80-99
	1:4	98	90-103

## Sensitivity

The minimum detectable dose of human IL-12 p40 is 3.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of IL-12 p40 in various matrices and diluted with the appropriate **Sample Diluent PT 1-ac** to produce samples with values within the dynamic range of the assay.

		Cell culture supernatants
1:2	Average% of Expected	105
	Range (%)	98-114
1:4	Average% of Expected	103
	Range (%)	88-121
1:8	Average% of Expected	99
	Range (%)	88-112
1:16	Average% of Expected	103
	Range (%)	80-116

## References

1. Picard C. et al. (2002). Am J Hum Genet. 70: 336-48.
2. Randolph AG. et al. (2004). Am J Hum Genet. 75: 709-15.
3. Tsunemi Y. et al. (2002). J Dermatol Sci. 30: 161-6.
4. provided by RefSeq, Jul 2008.