

Human IL-34 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human IL-34 concentrations in serum, plasma and human milk.

General Information

Catalogue Number	KE00151
Product Name	Human IL-34 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	146433
SwissProt	Q6ZMJ4

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-eg - 30 mL/bottle. For serum and plasma samples	1 bottle	
Sample Diluent PT 3-ec - 30 mL/bottle. For human milk	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	

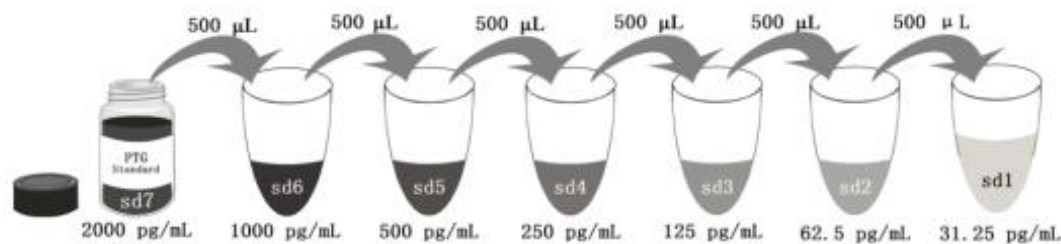
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-eg is for protein standard, serum and plasma samples.

Sample Diluent PT 3-ec is for protein standard and human milk.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 1-eg or PT 3-ec in 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-eg or PT 3-ec	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00151 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL34 ELISA kit is to be used to detect and quantify protein levels of endogenous IL34. The assay recognizes human IL34. An antibody specific for IL34 has been pre-coated onto the microwells. The IL34 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL34 is added to detect the captured IL34 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (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 34 (IL34) is a hematopoietic cytokine that acts as a key regulator of survival, proliferation and differentiation of myeloid lineage cells including monocytes, macrophages and osteoclasts. IL34 was identified in 2008 in a comprehensive proteomic analysis as a tissue-specific ligand of CSF-1 receptor (CSF-1R). This cytokine shares numerous common features with M-CSF, especially its receptor (M-CSF-R) explaining partly their functional overlap. IL34 is expressed in various tissues and it is most abundant in the spleen. Upon inflammation, other cells, such as fibroblasts and articular synovial cells, upregulate IL34 expression. Recent studies have reported that IL34 levels are elevated in the serum, synovial fluid, and synovial tissue of RA patients, and like CSF-1, IL34 is induced by TNF and IL-1beta in RA fibroblast-like synoviocytes (FLS).

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for serum or plasma. 1:4 or 1:8 dilution is recommended for human milk.

Safety Notes

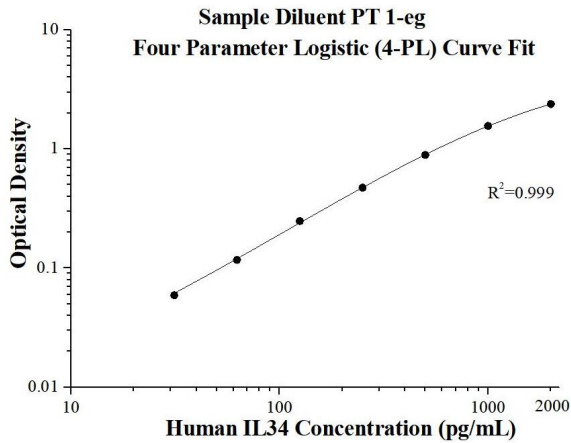
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

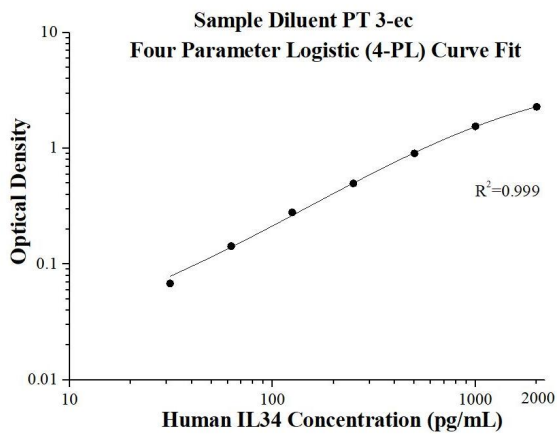
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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	0.049 0.041	0.045	-
31.25	0.111 0.097	0.104	0.059
62.5	0.166 0.157	0.162	0.117
125	0.305 0.280	0.293	0.248
250	0.530 0.506	0.518	0.473
500	0.952 0.919	0.936	0.891
1000	1.633 1.578	1.606	1.561
2000	2.436 2.420	2.428	2.383



(pg/mL)	O.D	Average	Corrected
0	0.096 0.094	0.095	-
31.25	0.158 0.168	0.163	0.068
62.5	0.237 0.239	0.238	0.143
125	0.375 0.374	0.375	0.280
250	0.593 0.592	0.593	0.498
500	0.997 1.002	1.000	0.905
1000	1.645 1.653	1.649	1.554
2000	2.373 2.395	2.384	2.289

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				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	1056.3	45.3	4.3
2	20	239.3	11.8	4.9
3	20	56.0	3.8	6.8

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1022.2	75.4	7.4
2	24	234.0	21.5	9.2
3	24	53.3	5.2	9.8

Recovery

The recovery of IL34 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 (%)
Human plasma	1:2	99	90-107
	1:4	105	100-113
Human milk	1:8	115	100-123
	1:16	110	96-130

Sample Values

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=16)	50.9	20.4-85.7
Human plasma (n=16)	49.7	29.6-76.3
Human milk (n=6)	4,041.2	3,276.9-5,438.5

Sensitivity

The minimum detectable dose of human IL34 is 9.7 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, human plasma samples were spiked with high concentrations of IL34 in various matrices and diluted with the appropriate **Sample Diluent PT 1-eg** to produce samples with values within the dynamic range of the assay. Human milk samples were diluted with the appropriate **Sample Diluent PT 3-ec** to produce samples with values within the dynamic range of the assay. (The human milk samples were initially diluted 1:2)

		Human plasma (PT 1-eg)	Human milk (PT 3-ec)
1:2	Average% of Expected	100	100
	Range (%)	94-108	-
1:4	Average% of Expected	108	105
	Range (%)	107-109	103-106
1:8	Average% of Expected	110	103
	Range (%)	102-117	95-110
1:16	Average% of Expected	113	92
	Range (%)	108-116	83-101

References

1. Menten P. et al. (2002) Cytokine Growth Factor Rev. 13(6):455-81.
2. Wang Y. et al. (2012) Nat Immunol. 13(8):753-60.
3. Chemel M. et al. (2012) Ann Rheum Dis. 71(1):150-4.
4. Baud'huin M. et al. (2010) J Pathol. 221(1):77-86.
5. Hwang SJ. et al. (2012) Arthritis Res Ther. 14(1):R14.
6. Tian Y. et al. (2013). J Interferon Cytokine Res. 33(7):398-401.
7. Moon SJ. et al. (2013). J Rheumatol. 40(11):1842-9.