Speedy™ Human IL-4 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50018

Size: 96T

Sensitivity: 1.9 pg/mL

Range: 31.25-2000 pg/mL

Usage: For the quantitative detection of human/cynomolgus monkey IL-4 concentrations in serum, plasma and cell culture supernatant.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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Table of content page

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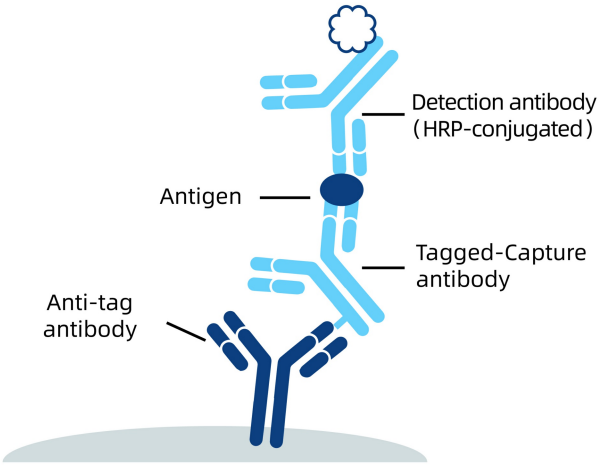
1. Background

Interleukin-4 (IL-4), a member of the α-helical cytokine family, is produced by activated CD4 + T cells, basophils, and mast cells. It promotes the proliferation and differentiation of antigen presenting cells. IL-4 also plays a pivotal role in antibody isotype switching and stimulates the production of IgE. This cytokine has been applied in the treatment of autoimmune disorder like multiple myeloma, cancer, psoriasis, and arthritis. IL-4 has also been extensively applied to inhibit detrimental effect of Th1. It may promote the growth of epithelial tumors by mediating increased proliferation and survival.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	12 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	1 bottles	
Capture antibody (100×) - 60uL/vials	60 vial	
Detection antibody, HRP-Conjugated (100×) - 60uL/vials	60 vial	
Additional Diluent AT-50018 - 6mL/bottle	6 bottle	
Sample Diluent PT 3-ef - 30mL/bottle	30 bottle	
Sample Diluent PT 4B1 - 30mL/bottle	30 bottle	
Detection Diluent - 15mL/bottle	15 bottle	
Wash Buffer Concentrate (20×) - 30mL/bottle	30 bottle	
Tetramethylbenzidine Substrate (TMB) - 12mL/bottle	12 bottle	
Stop Solution - 12mL/bottle	12 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

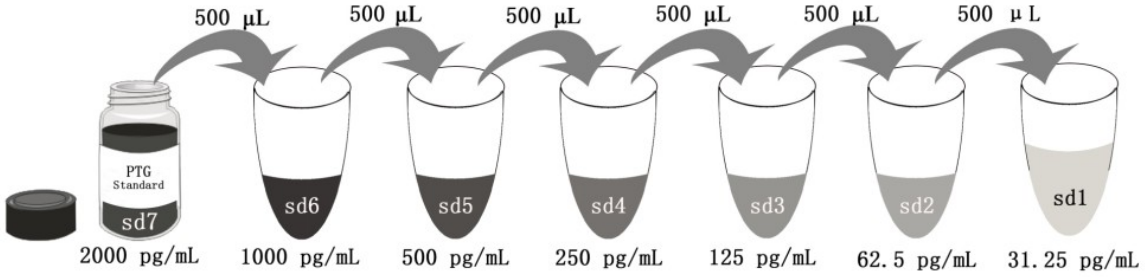
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 µL of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for human serum, human plasma, cynomolgus monkey serum, cynomolgus monkey plasma and cell culture supernatant.

7.4 Standard Serial Dilution:

For serum and plasma, add 2 mL Sample Diluent PT 3-ef in protein standard. For cell culture supernatant, add 2 mL Sample Diluent PT 4B1 in protein standard.



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 3-ef or PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group;

For serum or plasma, add 25 µL of Additional Diluent to the appropriate wells (No need incubation and wash);

For cell culture supernatant, no need to add Additional Diluent, directly follow the next step.

8.3 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.4 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.5 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.6 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.7 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.8 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.9 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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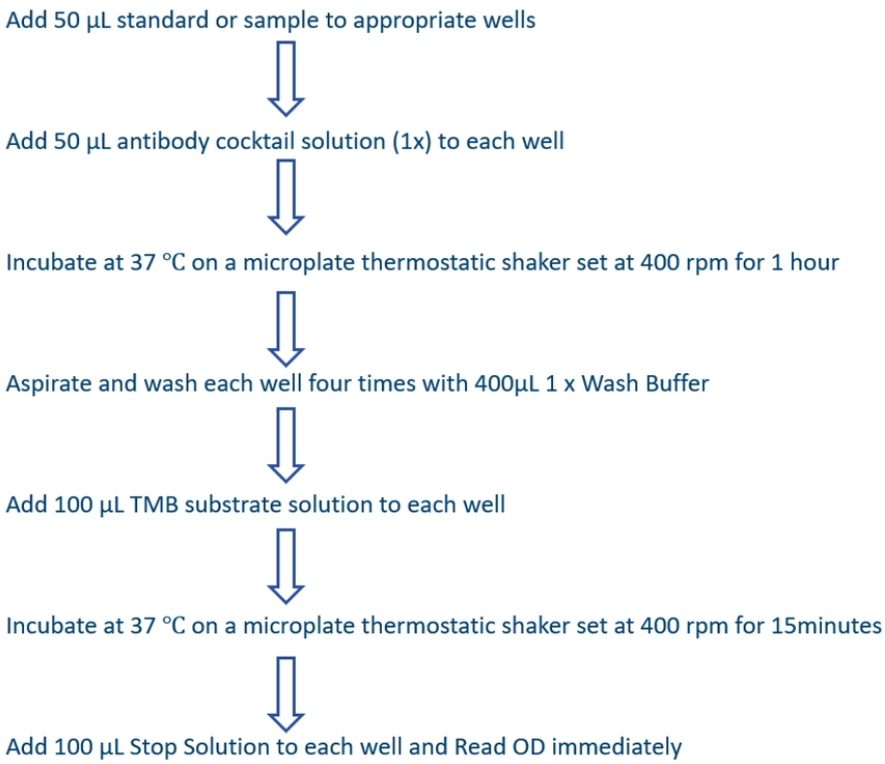

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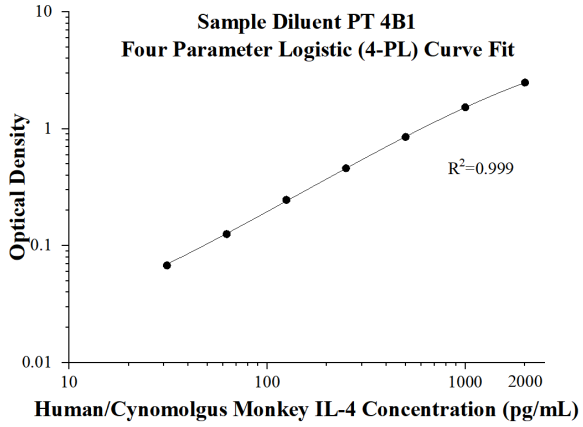
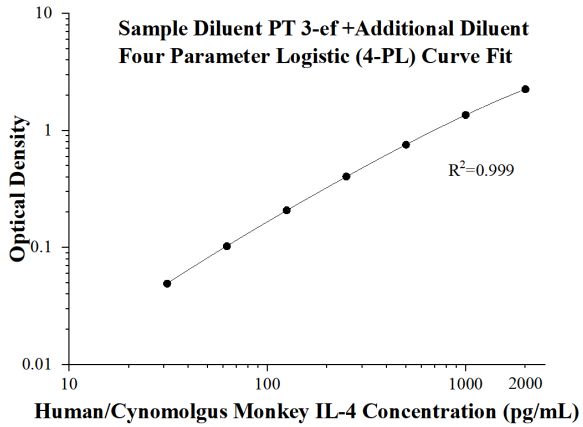
9. Validation Data

9.1 Standard curve

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	0.028 0.0306	0.0293	-
31.25	0.0922 0.1019	0.09705	0.06775
62.5	0.1515 0.1582	0.15485	0.12555
125	0.269 0.2829	0.27595	0.24665
250	0.4844 0.4932	0.4888	0.4595
500	0.8709 0.8926	0.88175	0.85245
1000	1.5489 1.5635	1.5562	1.5269
2000	2.5101 2.5245	2.5173	2.488



(pg/mL)	O.D	Average	Corrected
0	0.0166 0.0208	0.0187	-
31.25	0.0693 0.0664	0.06785	0.04915
62.5	0.1221 0.1208	0.12145	0.10275
125	0.2283 0.2258	0.22705	0.20835
250	0.4233 0.4225	0.4229	0.4042
500	0.7796 0.7723	0.77595	0.75725
1000	1.3892 1.3682	1.3787	1.36
2000	2.284 2.271	2.2775	2.2588

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,092.3	18.7	1.7
2	8	253.7	7.8	3.1
3	8	120.6	2.1	1.8

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	1,069.6	35.1	3.3
2	16	249.7	8.1	3.2
3	16	118.1	3.5	3.0

9.3 Recovery

The recovery of human/cynomolgus monkey IL-4 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	86	80-93
Cynomolgus monkey serum	1:2	91	89-93
	1:4	104	103-106
Cell culture supernatant	1:2	107	90-116

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9.4 Sample values

Human plasma - Fifteen samples were evaluated for detectable levels of IL-4 in this assay. Fifteen measured less than the lowest IL-4 standard, 31.25 pg/mL.

Cynomolgus monkey serum - Eight samples were evaluated for detectable levels of IL-4 in this assay. Eight measured less than the lowest IL-4 standard, 31.25 pg/mL.

Cell Culture Supernatant - Human peripheral blood mononuclear cells (0.5 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 5 µg/mL PHA and 10 ng/mL recombinant human IL-2. After 7 days of culture, cells were changed into DMEM with 10% fetal bovine serum, 10 ng/mL PMA, 1 µg/mL Ca^{2+} ionophore and 5 µg/mL PHA, and cultured for 1 day. An aliquot of the culture supernates were removed, assayed for IL-4.

Condition	IL-4 (pg/mL)
Unstimulated	ND
Stimulated	278.4

ND*=Non-detectable

Cynomolgus monkey peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate. Cells were stimulated with 50 ng/mL PMA and 1 ug/mL Ionomycin for 1 day. An aliquot of the culture supernates were removed, assayed for IL-4.

Condition	Day 1 (pg/mL)
Unstimulated	ND
Stimulated	648.6

ND*=Non-detectable

9.5 Sensitivity

The minimum detectable dose of human/cynomolgus monkey IL-4 is 1.9 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, human plasma and cynomolgus monkey serum samples were spiked with high concentrations of human/cynomolgus monkey IL-4 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma (Sample Diluent PT 3-ef)	Cynomolgus monkey serum (Sample Diluent PT 3-ef)	Cell culture supernatant (Sample Diluent PT 4B1)
1:2	Average% of Expected	89	81	100
	Range (%)	88-89	81-82	-
1:4	Average% of Expected	109	104	113
	Range (%)	105-112	102-106	104-122
1:8	Average% of Expected	101	102	112
	Range (%)	100-102	97-106	107-117
1:16	Average% of Expected	110	99	118
	Range (%)	109-111	94-104	107-129

9.7 Calibration

The NIBSC/WHO Human Total IL-4 Reference Reagent (11/82) (rDNA derived), which was intended as a potency standard, was evaluated in this kit. The dose response curve of this Reference Reagent parallels the Proteintech standard curve. To convert sample values obtained with the Authentikine Human Total IL-4 ELISA kit to approximate NIBSC/WHO (11/82) values, use the equation below.

NIBSC (11/82) approximate value (IU/mL)=0.011× Proteintech Human Total IL-4 value (pg/mL)

9.8 Specificity

This assay recognizes natural and recombinant human/cynomolgus monkey IL-4.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Recombinant mouse:	Recombinant rat:
IL-4 Rα	IL-13	IL-4
GM-CSF	GM-CSF	
gp130	IL-4	
IL-5		

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10. References

1. Dhanda SK. et al.(2013) Clin Dev Immunol.

2. Müller-Hermelink N. et al. (2008) Cancer Cell.13: 507-18.

3. Lebman DA. et al. (1988) 168: 853-62.

4. provided by RefSeq, Jul 2008.

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