Rat IL-10 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE20022

Size: 96T

Sensitivity: 6.0 pg/mL

Range: 31.25-2000 pg/mL

Usage: For the quantitative detection of rat IL-10 concentrations in serum, plasma, cell culture supernatant, tissue lysate and tissue homogenate.

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

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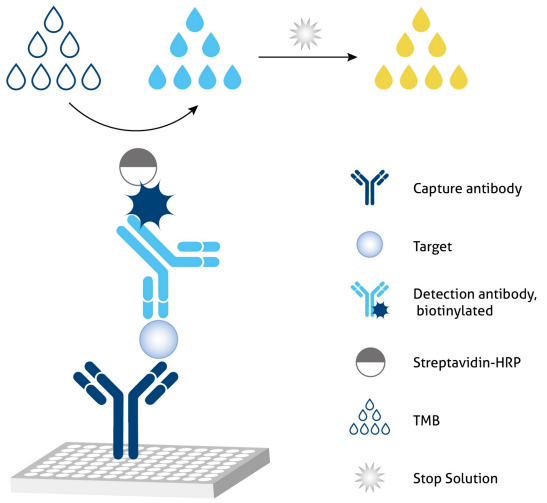
1. Background

IL-10 is an anti-inflammatory cytokine, produced by T helper (Th) cells, macrophages, monocytes, and B cells, that plays a crucial role in preventing inflammatory and autoimmune pathologies. It downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway. IL-10, along with its receptors, describes an important role in pathogenesis of various diseases, including infectious, inflammatory, autoimmune diseases.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns 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.

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4. Kit Components and 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 (100×) - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle. For rat serum, plasma and cell culture supernatant.	1 bottle	
Sample Diluent PT 4B1-ec - 30 mL/bottle. For tissue lysate and tissue homogenate.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 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

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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.

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

6.5 Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1 x PBS to remove excess blood, Then add 1 mL of 1 x PBS per 100 mg tissue, homogenized and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 10000 x g at 4°C. Collect the supernatant, assay immediately or aliquot and store 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 Detection Antibody(1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

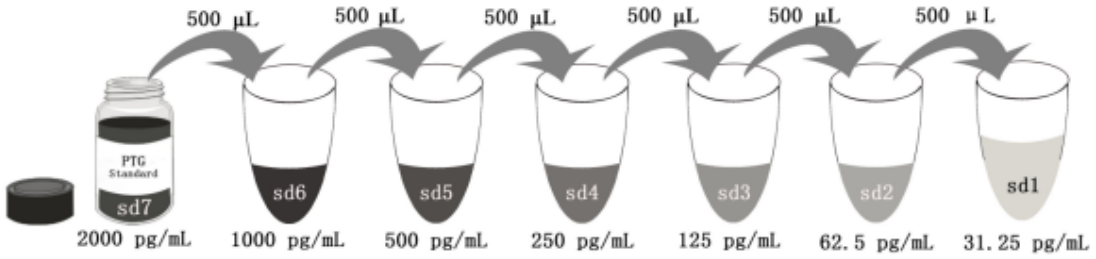
7.3 Streptavidin-HRP(1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, 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 or 1:4 is recommended for cell culture supernatant; 1:8 or 1:16 is recommended for tissue lysate and tissue homogenate.

7.5 Standard Serial Dilution:

For rat serum, plasma and cell culture supernatant, add 2mL Sample Diluent PT 4B1 in protein standard. For tissue lysate and tissue homogenate, add 2mL Sample Diluent PT4 B1-ec 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 4B1 or PT 4B1-ec	**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 and Streptavidin-HRP 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, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 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 1X 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.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 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, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

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.				

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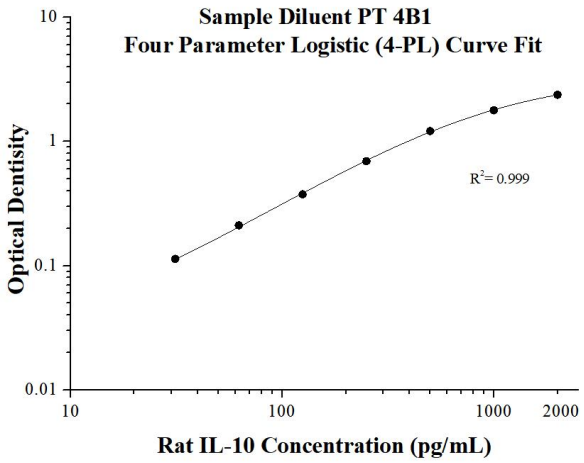
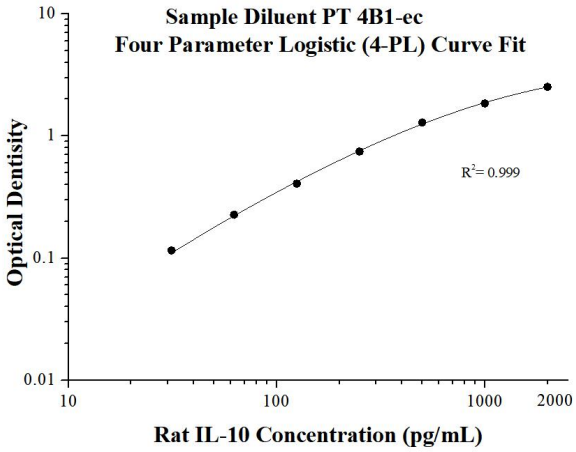
9. Validation Data

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	0.0765 0.0752	0.07585	-
31.25	0.1903 0.188	0.18915	0.1133
62.5	0.2882 0.2855	0.28685	0.211
125	0.4484 0.4528	0.4506	0.37475
250	0.7725 0.7682	0.77035	0.6945
500	1.2884 1.2893	1.28885	1.213
1000	1.8581 1.861	1.85955	1.7837
2000	2.4671 2.442	2.45455	2.3787



(pg/mL)	O.D	Average	Corrected
0	0.0674 0.0637	0.06555	-
31.25	0.1811 0.1814	0.18125	0.1157
62.5	0.3004 0.2839	0.29215	0.2266
125	0.4798 0.4656	0.4727	0.40715
250	0.8321 0.789	0.81055	0.745
500	1.3999 1.3063	1.3531	1.28755
1000	1.9327 1.8934	1.91305	1.8475
2000	2.6072 2.5741	2.59065	2.5251

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9.2 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	1,893.5	33.4	1.8
2	20	461.9	10.5	2.3
3	20	116.0	2.7	2.3

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,792.2	72.9	4.1
2	24	435.4	9.2	2.1
3	24	113.1	3.7	3.2

9.3 Recovery

The recovery of rat IL-10 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Rat serum	1:2	79	76-80
	1:4	86	76-104
Cell culture supernatant	1:2	103	82-114
	1:4	107	92-119
Tissue lysate	1:16	82	76-92
	1:32	91	86-94
Tissue homogenate	1:16	91	83-99
	1:32	111	98-120

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9.4 Sample values

Rat serum - Sixteen samples were evaluated for detectable levels of rat IL-10 in this assay. Sixteen measured less than the lowest rat IL-10 standard, 31.25 pg/mL.

Cell culture supernatant - Rat splenocytes (1 x 10^7 cells/mL) were cultured for 2 days in DMEM supplemented with 10% fetal bovine serum and stimulated with 5.0 µg/mL Concanavalin A. An aliquot of the cell culture supernatant was removed, assayed for rat IL-10 and measured 329 pg/mL.

Tissue lysate - Organs from 2-3 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized with a tissue homogenizer, and **Extraction Reagent** was added. Rat tissue lysate from rat heart tissue was removed, assayed for rat IL-10, and measured 753.8 pg/mL.

Tissue homogenate - Organs from 2 rats were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized in 5-10 mL of PBS in a tissue homogenizer, and stored at ≤ -80 °C 5-10 minutes. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 10 minutes at 5000 x g to remove particulate. Homogenates from heart tissue was assayed for rat IL-10 and measured 3,022.1 pg/mL.

9.5 Sensitivity

The minimum detectable dose of rat IL-10 is 6.0 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, rat serum samples were spiked with high concentrations of rat IL-10 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant samples, tissue lysates and tissue homogenates were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.
(The tissue lysate was initially diluted 1:4. The tissue homogenate was initially diluted 1:4.)

		Rat serum (Sample Diluent PT 4B1)	Cell culture supernatant (Sample Diluent PT 4B1)	Tissue lysate (Sample Diluent PT 4B1-ec)	Tissue homogenate (Sample Diluent PT 4B1-ec)
1:2	Average% of Expected	91	100	100	100
	Range (%)	73-101	-	-	-
1:4	Average% of Expected	88	98	103	115
	Range (%)	82-98	91-110	93-115	109-124
1:8	Average% of Expected	86	96	112	109
	Range (%)	76-100	87-106	101-118	104-111
1:16	Average% of Expected	89	105	-	83
	Range (%)	74-113	94-113	-	81-84

10. References

1. Glocker, E., et al. IL-10 and IL-10 receptor defects in humans. ANN NY ACAD SCI. 1246: 102-107 (2011).
2. Eskdale, J., et al. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A. 95: 9465-70 (1998).
3. Malefyt, R., et al. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med. 174: 1209-20 (1991).

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