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EliKine™ Rat IL-4 ELISA Kit

Cat #: KTE9003 Size: 48 T/96 T

	Rat IL-4 ELISA Kit		
REF	**Cat #:** KTE9003	LOT	**Lot #:** Refer to product label
	Detection range: 2 pg/mL - 80 pg/mL		**Sensitivity:** 0.5 pg/mL
	Precision: Intra-assay Precision: The CV (%) < 10%. Inter-assay Precision :The CV (%) < 12%		**Recovery:** The recovery ranged from 98% to 116% with an overall mean recovery of 106%.
	Specificity: EliKine™ Rat IL-4 ELISA Kit has high sensitivity and excellent specificity for detection of Rat IL-4. No significant cross-reactivity or interference between Rat IL-4 and analogues was observed.		
	Applicable samples: Serum, Plasma, Tissue, Cells, Cell supernatant, and related Liquid Samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Interleukin 4 is a pleiotropic cytokine produced primarily by activated T lymphocytes, mast cells and basophils. IL-4 has multiple immune response-modulating functions on a variety of cell types. It is an important modulator of the differentiation of precursor T helper cells to the Th2 subset that mediates humoral immunity and modulates antibody production. EliKine™ Rat IL-4 ELISA Kit employs a double antibody sandwich method to quantitate Rat IL-4 in samples. An antibody specific for Rat IL-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Rat IL-4 present is bound by the immobilized antibody. After removing any unbound substances, a HRP-conjugated antibody specific for Rat IL-4 is added to the wells. After washing, remove any unbound HRP-conjugated antibody reagent, adding HRP Substrate (TMB), TMB turns blue under the catalysis of HRP, and turns yellow after adding stop solution. Measure the OD value with a microplate reader at 450 nm wavelength. The Rat IL-4 concentration is proportional to the OD450 nm value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Rat IL-4 Microplate	48 wells	96 wells	4℃
Rat IL-4 Standard (160 pg/mL)	0.5 mL	0.5 mL	4℃
Standard Diluent	1.5 mL	1.5 mL	4℃
HRP-conjugated Rat IL-4 Detect Antibody	3 mL	6 mL	4℃
Sample Diluent	3 mL	6 mL	4℃

HRP Substrate A	3 mL	6 mL	4℃, protected from light
HRP Substrate B	3 mL	6 mL	4℃, protected from light
Stop Solution	3 mL	6 mL	4℃
Wash Buffer	20 mL (20×)	20 mL (30×)	4℃
Plate Covers	1	2	RT

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm
- Multi channel pipette or automated microplate washer
- Incubator, refrigerated centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water

Reagent Preparation

Standard Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃. Standard Diluent is used for dilution of standard.

Sample Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃. Sample Diluent is used for dilution of samples.

Rat IL-4 standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

HRP-conjugated Rat IL-4 Detect Antibody: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

HRP Substrate A: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

HRP SubstrateB: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Stop Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃

1×Wash Buffer: Equilibrate to room temperature and dilute the Wash Buffer 20 times with deionized water at 48 T and 30 times with deionized water at 96 T to obtain 1×Wash Buffer before use. Mix gently to avoid foaming. Store at room temperature. Please note that 1×Wash buffer is stable for 30 days.

Standard curve setting: dilute 160 pg/mL standard with Standard Diluent to 80, 40, 20, 10, and 5pg/mL of Rat IL-4 standard just as below

NUM.	Volume of Standard	Volume of Standard Diluent (μL)	The Concentration of Standard（pg/mL）
Std.1	150 μL of 160 pg/mL	150	80
Std.2	150 μL of Std.1 (80 pg/mL)	150	40
Std.3	150 μL of Std.2 (40 pg/mL)	150	20
Std.4	150 μL of Std.3 (20 pg/mL)	150	10
Std.5	150 μL of Std.4 (10 pg/mL)	150	5

Note：Always prepare a fresh set of standards per use.

Sample Preparation

1. Cell culture supernatants: Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
2. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

3. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
4. Tissue: Weigh 0.1g of the tissue, add 0.9mL of cold 0.01 M pH7.4 PBS (protease inhibitors can be added), and homogenize in an ice bath. Collect the homogenate, centrifuge at 10,000 g at 4℃ for 10 min, take the supernatant and immediately measure or aliquot and store samples at -20℃ to avoid repeated freezing and thawing.
5. Cells: The adherent cells were gently washed with cold PBS, then digested with trypsin, centrifuged at 1,000 g for 5 min and collect cells; Suspended cells can be collected directly by centrifugation. The collected cells were washed 3 times with cold PBS. Add 150-200 μL of PBS to resuspend each 10^6 cells (it is recommended to add protease inhibitors to PBS); If the content is very low, the volume of PBS can be reduced) and then ultrasonic break in ice bath for 2 min (power 20% or 200 W, ultrasound for 3 seconds, interval 7 seconds, repeated 12 times). Centrifuge the extract at 4℃ and 10,000 g for 10 min. Take the supernatant and immediately measure or aliquot and store samples at -20℃ to avoid repeated freezing and thawing.
6. Urine, saliva, and other liquid samples: Centrifuge 3,000 g for 10 min, take the supernatant and immediately measure or aliquot and store samples at -20℃ to avoid repeated freezing and thawing.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 h, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal. The strips used for testing are equilibrated to room temperature before use.
2. Set Blank well (Blank well without sample, HRP conjugated Rat IL-4 Detect Antibody, all other steps are the same) and Test wells separately. Add 40 μL of Sample Diluent and 10 μL of sample (the final sample dilution is 5 times) to Test wells. Add the sample to the bottom of the enzyme-linked immunosorbent assay plate well, avoiding touching the well wall as much as possible, and gently shake and mix well. Add 50 μL of standard samples of different concentrations to the standard wells in sequence. Cover with the plate cover provided. Incubate for 30 min at 37℃.
3. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 μL) using a multi channel pipette or automated microplate washer, and let it stand for 1-2 min, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
4. Add 50 μL of HRP-conjugated Rat IL-4 Detect Antibody to each well, excluding Blank well. Cover with the plate cover provided. Incubate for 30 min at 37℃.
5. Repeat the wash as in step 3.
6. Add 50 μL of HRP Substrate A to each well first, then add 50 μL of HRP Substrate B, gently shake and mix well, and incubate for 15 min at 37℃. Protect from light.
7. Add 50 μL of Stop solution to each well. Stop Solution should be added to the plate in the same order as TMB. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 15 min, zero with blank well and measure the absorbance at 450 nm for each well.

Data Analysis

1. Calculate the average OD value of each standard and sample in multiple Wells.
2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Note: If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

Typical standard curve（$R^2 \geqslant 0.99$）

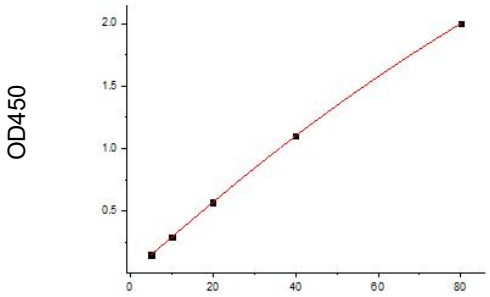

Figure1. Standard Curve of Rat IL-4 in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. If Standard Diluent and Sample Diluent appears to turn yellow or a small amount of precipitation, etc. Please centrifuge to remove the precipitate, which will not affect normal use.
2. Do not mix or substitute reagents with those from other lots or sources.
3. 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.
4. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
5. Stop Solution has certain Corrosive. Please take protective measures when operating.

Recommended Products

Catalog No.	Product Name
KTE6015	EliKine™ Human IL-4 ELISA Kit
KTE9004	EliKine™ Rat IL-6 ELISA Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.