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EliKine™ Rat IL-2 ELISA Kit

Cat #: KTE9002 Size: 48 T/96 T

	Rat IL-2 ELISA Kit		
REF	**Cat #:** KTE9002	LOT	**Lot #:** Refer to product label
	Detection range: 100 pg/mL-1,200 pg/mL		**Sensitivity:** 80 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-2 ELISA Kit has high sensitivity and excellent specificity for detection of Rat IL-2. No significant cross-reactivity or interference between Rat IL-2 and analogues was observed.		
	Applicable samples: Tissues, Serum, Plasma, Cell culture supernatants and other liquid samples		
	Storage: Stored at 4℃ for 6 months		

Assay Principle

Interleukin 2 (IL-2) is a pleiotropic cytokine produced primarily by mitogen- or antigen-activated T lymphocytes. IL-2 plays a key role in promoting the clonal expansion of antigen-specific T cells. In addition, IL-2 has also been shown to mediate multiple immune responses on a variety of cell types. EliKine™ Rat IL-2 ELISA Kit employs a double antibody sandwich method to quantitate Rat IL-2 in samples. An antibody specific for Rat IL-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Rat IL-2 present is bound by the immobilized antibody. After removing any unbound substances, a HRP-conjugated antibody specific for Rat IL-2 is added to the wells, form an antibody-antigen-enzyme-labeled antibody complex. Following a wash to remove any unbound streptavidin-enzyme 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 IL-2 concentration is proportional to the OD450 nm value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Rat IL-2 Microplate	48 wells	96 wells	4℃
Rat IL-2 Standard (1,800 pg/mL)	0.5 mL	0.5 mL	4℃
Standard Diluent	1.5 mL	1.5 mL	4℃
Sample Diluent	3 mL	6 mL	4℃
Rat IL-2 Detect Antibody	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	5 mL	10 mL	4℃
Wash Buffer	20 mL (20×)	20 mL (30×)	4℃
Plate Covers	2	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℃.

Sample Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Rat IL-2 Standard (1,800 pg/mL): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Rat IL-2 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 Substrate B: 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℃.

Wash Buffer: Equilibrate to room temperature. For 48 T, dilute Wash Buffer with deionized water 1:20 to obtain the 1×Wash Buffer before use, for 96 T, dilute Wash Buffer with deionized water 1:20 to obtain the 1×Wash 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 1,800 pg/mL standard with Standard Diluent to 1,200, 800, 400, 200 and 100 pg/mL of Rat IL-2 standard just as below.

NUM.	Volume of Standard	Volume of Standard Diluent (μL)	The Concentration of Standard（pg/mL）
Std.1	200 μL of 1,800 pg/mL	100	1,200
Std.2	200 μL of Std.1 (800 pg/mL)	100	800
Std.3	100 μL of Std.1 (400 pg/mL)	100	400
Std.4	100 μL of Std.4 (200 pg/mL)	100	200
Std.5	100 μL of Std.5 (100 pg/mL)	100	100

Note：Always prepare fresh 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. Tissues: Weigh 0.1 g tissues, add 1 mL 0.01M PBS pH7.4 (Proteinase inhibitors can be added) and homogenize on ice.

Centrifuge at 3,000 g for 20 min at 4℃. Use supernatant for assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

5. Urine and other liquid biological samples:Centrifuge at 3,000 g for 10min, Use supernatant for assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

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. Add Sample: Blank well (Blank Well without sample, Rat IL-2 Detect Antibody, all other steps are the same). Test Well for sample, first add 40 µL of Sample Diluent to the well, then add 10 µL of sample. Add the sample to the bottom of the ELISA 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 5 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 Rat IL-2 detect antibody to each 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 . Cover the plate 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 30 min, using a microplate reader set to 450 nm.

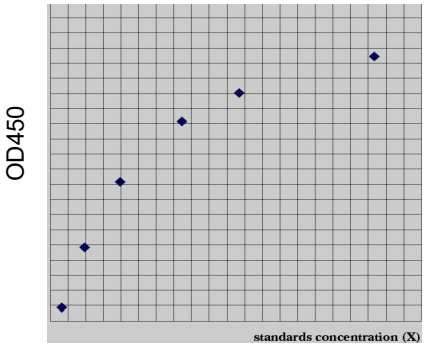
Data Analysis

1. Average the duplicate readings for each standard and sample and subtract the the blank wells optical density (O.D.).
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 (×n×5).

Typical Data

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



Rat IL-2 standard (pg/mL)

Figure1. Standard curve of Rat IL-2 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., it is caused by the serum contained in the reagent. 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
KTE6013	EliKine™ Human IL-1β ELISA Kit
KTE7005	EliKine™ Mouse IL-1β ELISA Kit

Disclaimer

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