Human IL-2 Uncoated ELISA Kit

Catalog NO.:RK04265

version: 2.0

This package insert must be read in its entirety before using this product

Introduction

The Uncoated ELISA Kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IL-2 in human serum, plasma and cell culture supernatants.The Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples.

Material Provided & Storage Conditions

The unopened kit can be stored for less than two weeks at 2-8 ℃. However, for long time storage, keep the components of the kit according to the instructions. After the kit is opened once, it is only recommended to be used within one month. Don't use the kit after the expiry date mentioned on the box.

Part	Size			Concentration	Storage of opened/reconstituted material
	5set	15set	20set		
Human IL-2 Capture Antibody	1×200ul	1×600 μL	1×800ul	1 mg/mL	May be stored for up to 6 months at -20℃.*
Human IL-2 Biotin-Conjugate Antibody	1×100ul	1×300 μL	1×400ul	25 μg/mL	May be stored for up to 6 months at -20℃.*
Human IL-2 Standard Lyophilized	1× 2ng/vial	1× 2ng/vial	2× 2ng/vial	/	May be stored for up to 6 months at -20℃.*
Streptavidin-HRP Concentrated (200×)	1x350ul	1x1000 μL	2x700ul	/	May be stored for up to 6 months at 2-8℃.*DO NOT FREEZE

Specification	1

Other Solution Required

Reagent Kit (1set)

Part	Size	Cat. No.	Storage of opened/reconstituted material
uncoated plate	1 x 96T	RM01758	May be stored for up to 6 months at room temperature*
Coating Buffer	1 x 20 mL	RM01756	May be stored for up to 6 months at 2-8℃.*
Blocking Buffer	1 x 20 mL	RM01757	May be stored for up to 6 months at 2-8℃.*
Standard/Sample Diluent	1 x 20 mL	RM00023	May be stored for up to 6 months at 2-8℃.*
Biotin-Conjugate Antibody Diluent	1 x 12 mL	RM00024	May be stored for up to 6 months at 2-8℃.*
Streptavidin-HRP Diluent	2 x 12 mL	RM00025	May be stored for up to 6 months at 2-8℃.*
Wash Buffer(20x)	1 x 30 mL	RM00026	May be stored for up to 6 months at 2-8℃.*
TMB Substrate	1 x 12 mL	RM00027	May be stored for up to 6 months at 2-8℃.*
Stop Solution	1 x 6 mL	RM00028	May be stored for up to 6 months at 2-8℃.*
Plate Sealers	60 x 1pcs	RM01759	May be stored for up to 6 months at room temperature*

1. It's recommended to diluent Wash Buffer(20x) with Ultra-pure water.

Some components list above may be preparation separately:

Coating buffer(PBS): 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2-7.4, 0.2 μm filtered.

Blocking Buffer: 1% BSA, 5% sucrose in PBS, pH 7.2-7.4, 0.2 μm filtered.

Standard/Sample Diluent/Biotin-Conjugate Antibody Diluent/Streptavidin-HRP Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered.

Wash Buffer:0.05% Tween® 20 in PBS, pH 7.2-7.4

TMB Substrate:1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) .

Stop Solution:1 N HCL

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator
6. Test tubes for dilution of standards and samples

Precautions

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Please perform simple centrifugation to collect the liquid before use.
7. Do not mix or substitute reagents with those from other lots or other sources.
8. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
9. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
10. Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.
11. Reuse of dissolved standard is not recommended.
12. The kit should not be used beyond the expiration date on the kit label.
13. The kit should be away from light when it is stored or incubated.
14. To reduce the likelihood of blood-borne transmission of infectious agents,

handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

15. To avoid cross contamination, please use disposable pipette tips.
16. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
17. This assay is designed to eliminate interference by other factors present in biological samples.
18. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing the correlated IgG as in this kit may interfere with this assay.

Cell Culture supernatants: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Serum : Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma : Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of coolection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Note : It is suggested that all samples in a study be collected at the same time of the day.Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

Reagent Preparation

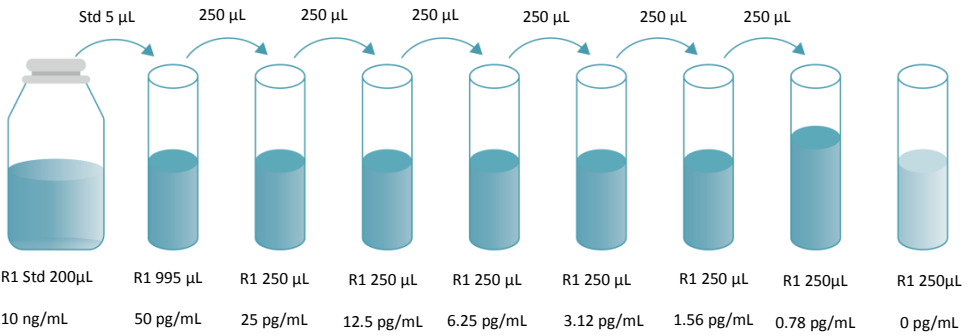
Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Human IL-2 Capture Antibody - It's recommended to coat at a concentration of 4 μg/mL with Coating Buffer. Add 100 μL of the diluted Capture Antibody to a uncoated plate each well. Incubate overnight at 4℃. Aspirate each well and block plates by adding 200 μL of Blocking Buffer to each well. Incubate for 2 hours at

37℃. Aspirate each well and add wash buffer 350 μL/well. Aspirate each well after holding 40 seconds,repeating the process two times for a total of three washes.

Standard - Reconstitute the Standard Lyophilized with 0.2 mL Standard/Sample Diluent. This reconstitution produces a stock solution of 10 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use the standard stock to produce a dilution series (below) with Standard/Sample Diluent. Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0pg/mL). Use diluted standards within 20 minutes of preparation.The remaining standards are aliquoted and stored at -20 to -70℃.



Human IL-2 Biotin-Conjugate Antibody - It's recommended to use at a 500-fold dilution with Biotin-Conjugate Antibody Diluent. Note: The diluted working solution should be used within 30 minutes.

Streptavidin-HRP Concentrated(200×) - It is recommended to dilute 1:200 of Concentrated Streptavidin-HRP (200x) with Streptavidin-HRP Diluent before use.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 100 µL Standard/sample Diluent (R1) in a blank well.
3. Add 100 µL different concentration of standard or sample in other wells, Cover with the adhesive strip provided. Incubate for 2 hours at 37℃. record the plate layout of standards and sample assay.
4. Prepare the Human IL-2 Biotin-Conjugate Antibody Working Solution 15 minutes early before use.
5. Add wash buffer 350 µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
6. Add 100 µL Working Human IL-2 Biotin-Conjugate Antibody in each well, cover with new adhesive strip provided. Incubate for 1 hour at 37℃.
7. Prepare the Streptavidin-HRP Concentrated (200X) Working Solution 15minutes early before use.
8. Repeat the aspiration/wash as in step 5.
9. Add 100 µL Working Streptavidin-HRP in each well, cover with new adhesive

strip provided.Incubate for 0.5 hour at 37℃.

10. During the incubation, turn on the microplate reader to warm up.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL TMB Substrate to each well. Incubate for 15-20 minutes at 37℃ .Protect from light.
13. Add 50 µL Stop Solution, determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Assay Procedure Summary

Prepare the standard and reagents

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Add 100μL of standards or test samples to each well

Incubate for 2 hours at 37℃, then wash 3 times

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Add 100μL Working Human IL-2 Biotin-Conjugate Antibody

Incubate for 1 hour at 37℃, then wash 3 times

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Add 100μL Working Streptavidin-HRP

Incubate for 0.5 hour at 37℃, then wash 3 times

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Add 100μL TMB Substrate

Incubate for 15-20 min at 37℃ under dark condition

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Add 50μL Stop Solution

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Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

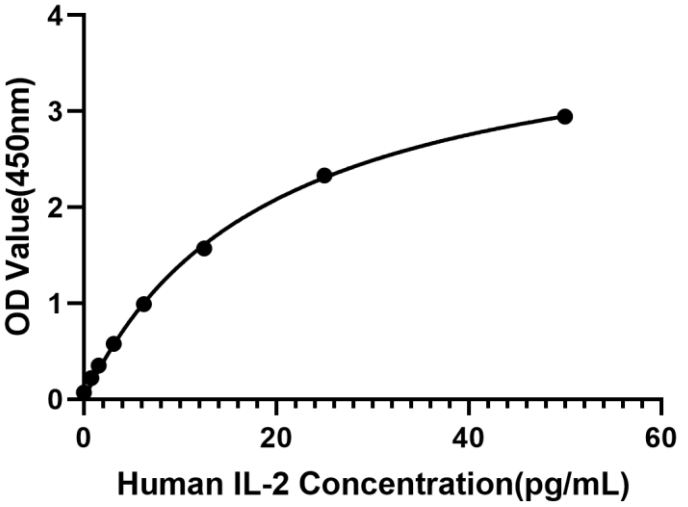
Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).

2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve. The data may be linearized by plotting the IL-2 concentrations versus the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of IL-2 assayed.

Specificity

This assay recognizes both recombinant and natural human IL-2. The factors listed below were prepared at 10ng/mL and assayed for cross-reactivity. No significant cross-reactivity was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between IL-2 and all the analogues, therefore, cross reaction may still exist.

Trouble Shooting

*For research purposes only. Not for therapeutic or diagnostic purposes.

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.
	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.