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USER MANUAL



ELISA KIT

ELISA Kit for Erythropoietin (EPO)

Catalog No.tcue31 96 Tests

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

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ELISA Kit for Erythropoietin (EPO)
Catalog No.tcue31

Available Sizes

Size: 96 Tests

Specifications

Research Area:	Endocrinology,Cytokine,Hormone metabolism,Hematology Reseach
Species Reactivity:	Human (homo sapiens)
Sample Type:	Serum, plasma and Tissue Homogenates, other biological fluids
Sensitivity:	12.1pg/ml
Recovery:	Recovery ranges are from 93-105% .
Detection Range:	31.2-2,000pg/mL
Assay Time:	3 hrs
Detection Method:	Sandwich ELISA, Double Antibody,Colorimetric; absorbance at 450 nm
Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Erythropoietin (EPO) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level EPO were tested on 3 different plates, 8 replicates in each plate. Intra-assay coefficient of variation (CV) <8%. Inter-assay CV <10%.

Test Principle

The microplate provided in this kit has been pre-coated with an antibody specific to erythropoietin. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to erythropoietin. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain erythropoietin, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of erythropoietin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

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Materials and Storage.

Store kit components at -20 °C unless specified otherwise.
DO NOT USE past kit expiration date. Some vials contain a small amount of reagents.
Spin tubeson pulse setting prior to opening

Components

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000μL
- Multi-channel pipette calibrated to deliver 50-250μL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm.

Safety Precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Oraliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

☐ Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

☐ Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

☐ Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

☐ Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

☐ Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.

☐ Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note: Samples to be used within 5 days may be stored at 4°C,otherwise samples must be stored at -20°C (≤1 month) or -80°C(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

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Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

☐ High target protein concentration (200-2000ng/ml): Dilution: 1:100. (i.e. Add 1μl of sample into 99 μl of Sample / Standard dilution buffer.)
☐ Medium target protein concentration (20-200ng/ml): Dilution: 1:10.(i.e. Add 10 μl of sample into 90 μl of Sample / Standard dilution buffer.)
☐ Low target protein concentration (0.312-20ng/ml): Dilution: 1:2.(i.e. Add 50 μl of sample into 50 μl of Sample / Standard dilution buffer.)
☐ Very low target protein concentration (≤0.312ng/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

1, Wash Buffer:

Dilute 20mL of Concentrated Wash Buffer (30X) into 580 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard:

1). 2000 pg/ml of standard solution: Add 0.5 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.
2). 2000 png/ml→31.2pg/ml of standard solutions: Label 6 Eppendorf tubes with 1000pg/ml,500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, respectively. Aliquot 250 ul of the Sample / Standard dilution buffer into each tube. Add 250 ul of the above 2000pg/ml standard solution into 1st tube and mix thoroughly. Transfer 250 ul from 1st tube to 2nd tube and mix thoroughly. Transfer 250 ul from 2nd tube to 3rd tube and mix thoroughly, and so on.
And the last EP tubes with Standard Diluent is the blank as 0pg/mL.

Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

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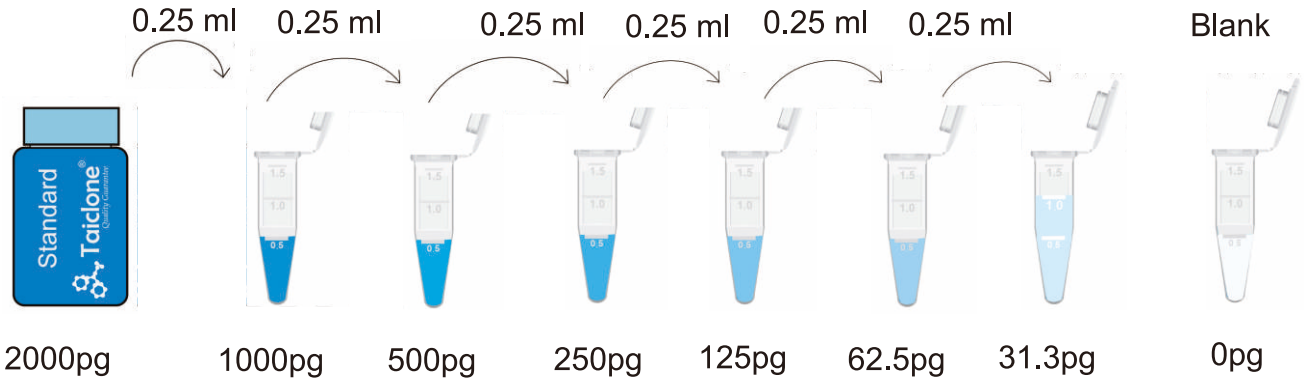
3. Detection Reagent A and Detection Reagent B - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again



NOTE:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standards within 15 minutes before assay. Please do not dissolve the reagents at 37oC directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL for one pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

This kit is compatible with EDTA-plasma, heparin plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

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Assay Procedure:
1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.
2. Remove the liquid of each well, don't wash.
3. Add 100μL of Detection Reagent A working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37°C.
4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100μL of Detection Reagent B working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37°C.
6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
7. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

Note:
1. Assay preparation: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20oC.
2. Samples or reagents addition：Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall.
For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. Please protect it from light.
7. The environment humidity which is less than 60% might have some effects on the final performance, therefore, a humidifier is recommended to be used at that condition.

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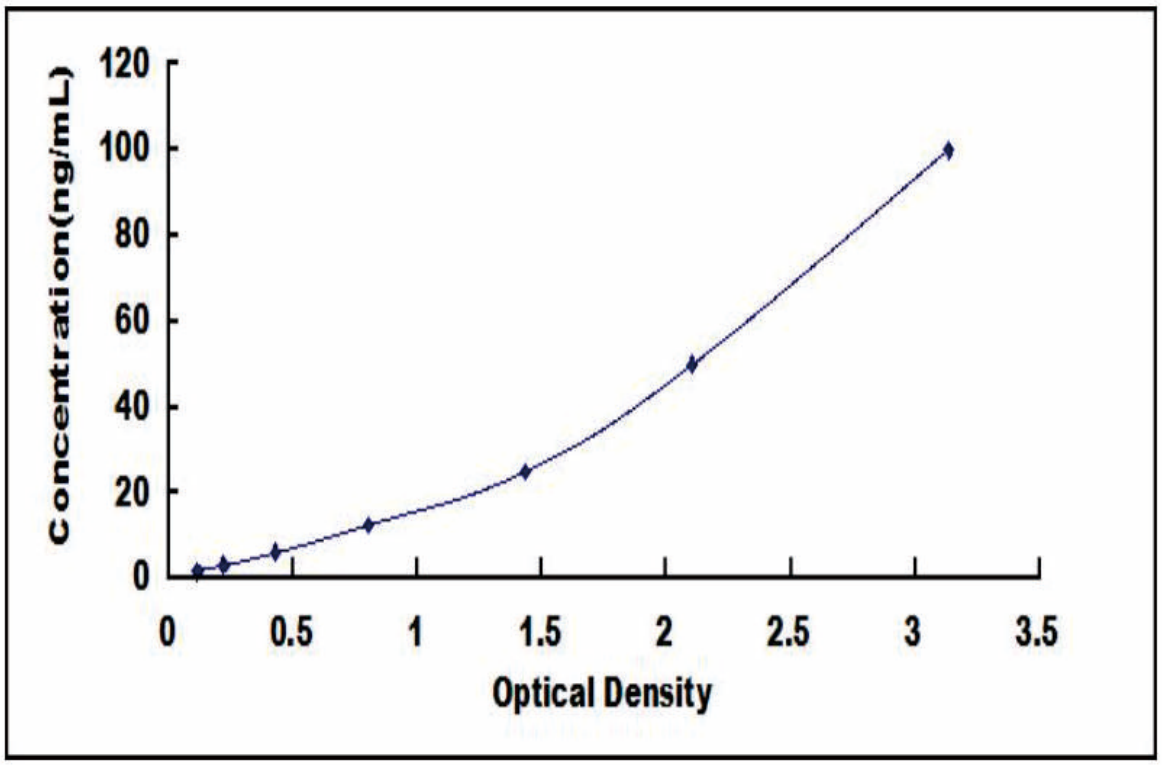
Summary

1. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Add 100μL standard or sample to each well for 90 minutes at 37°C
3. add 100μL Biotin- detection antibody working solution to each well for 60 minutes at 37°C
4. Aspirate and wash 3 times
5. Add 100μL Reagent B solution to each well. Incubate for 30 minutes at 37°C
6. Aspirate and wash 5 times
7. Add 90μL TMB substrate. Incubate 10 -20 minutes at 37°C
8. Add 50μL Stop Solution. Read at 450nm immediately
9. Calculation of results

Typical Data & Standard Curve

Results of a typical standard run of a eNOS ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	ng/ml	0	0.312	0.625	1.25	2.5	5	10	20
Y	OD450	0.076	0.149	0.222	0.385	0.67	1.097	1.964	2.462



Typical Standard Curve

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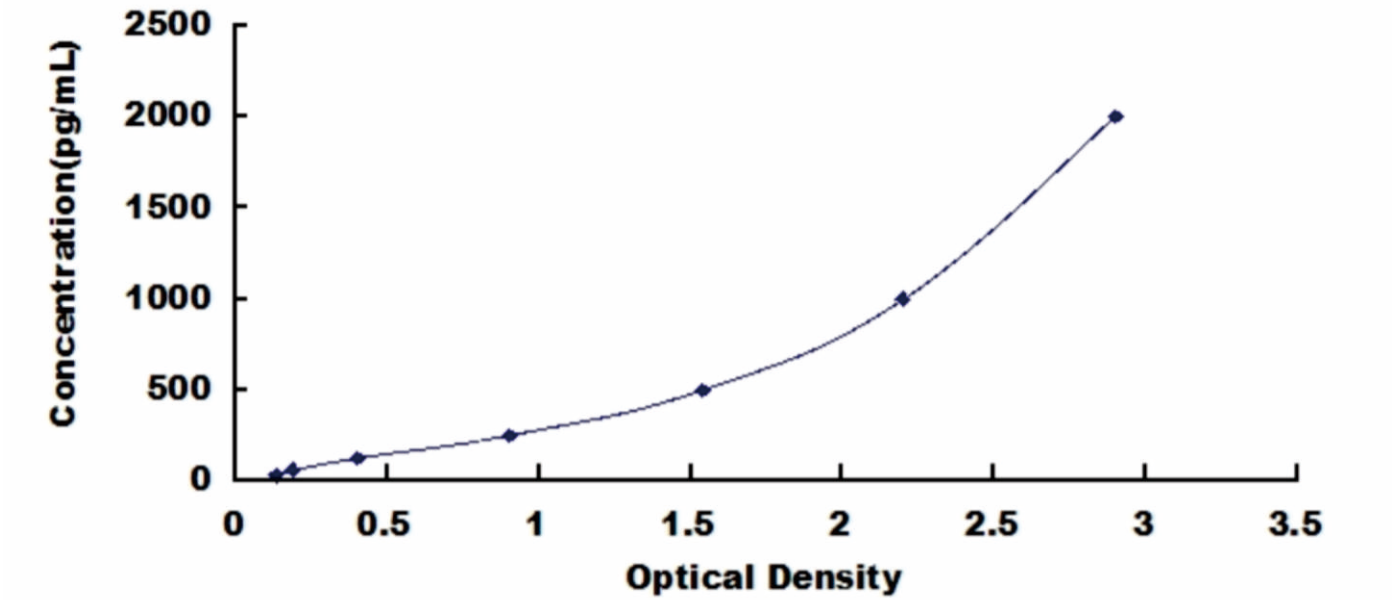
Calculation of result.

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with erythropoietin concentration on the y-axis and absorbance on the x-axis. Using some plot software, for instance, curve expert 1.30, is also recommended.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable.
However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), plotting log of the data to establish standard curve for each test is recommended. Typical standard curve below is provided for reference only.



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