Datasheet for ABIN1873508

IL-6 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IL-6 (IL6)
Reactivity:	Cow
Method Type:	Sandwich ELISA
Detection Range:	1.56 pg/mL - 100 pg/mL
Minimum Detection Limit:	1.56 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IL6 in bovine serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of High Sensitive Interleukin 6 (IL6). No significant cross-reactivity or interference between High Sensitive Interleukin 6 (IL6) and analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between High Sensitive Interleukin 6 (IL6) and analogues was observed.

Product Details

Sensitivity:	0.45 pg/mL
Grade:	High Sensitivity
Components:	• Pre-coated, ready to use 96-well strip plate • Plate sealer for 96 wells • Standard Diluent • Assay Diluent A • Assay Diluent B • Stop Solution • Standard • Detection Reagent A • Detection Reagent B • TMB Substrate • Wash Buffer (30 x concentrate) • Instruction manual
Material not included:	• Microplate reader with 450 nm filter. • Precision single or multi-channel pipettes and disposable tips. • Eppendorf Tubes for diluting samples. • Deionized or distilled water. • Absorbent paper for blotting the microtiter plate. • Container for Wash Solution

Target Details

Target:	IL-6 (IL6)
Abstract:	IL6 Products
UniProt:	P26892
Pathways:	TLR Signaling, Hormone Transport, Negative Regulation of Hormone Secretion, Myometrial Relaxation and Contraction, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Regulation of Carbohydrate Metabolic Process, Autophagy, Cell RedoxHomeostasis, Cancer Immune Checkpoints, Inflammasome

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient

	samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time. • Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. • Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. • There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed. • Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. • Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. • Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. • Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.
Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	3 h

Application Details

Plate:	Pre-coated
Protocol:	The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to High Sensitive Interleukin 6 (IL6). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to High Sensitive Interleukin 6 (IL6). 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 High Sensitive Interleukin 6 (IL6), 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 High Sensitive Interleukin 6 (IL6) in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	• Bring all kit components and samples to room temperature (18-25 °C) before use. • **Standard** - Reconstitute the Standard with 1.0 mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 200 pg/mL. Please firstly dilute the stock solution to 100 pg/mL and the diluted standard serves as the highest standard (100 pg/mL). Then prepare 7 tubes containing 0.5 mL Standard Diluent and produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL, 3.12 pg/mL, 1.56 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL • **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with Assay Diluent A and B, respectively (1:100). • **Wash Solution** - Dilute 20 mL of Wash Solution concentrate (30×) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×). • **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again. Note: • Making serial dilution in the wells directly is not permitted. • Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly. • 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 10myL for once pipetting. • The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once. • If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature

	and mix gently until the crystals are completely dissolved. • Contaminated water or container for reagent preparation will influence the detection result.
Sample Collection:	**Serum:** Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles. **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.01 mol/L,pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in 5-10mL of PBS with a glass homogenizer on ice(Micro Tissue Grinders woks, too). The resulting suspension was sonicated with an ultrasonic cell disrupter or subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 5 minutes at 5000×g. Remove the supernate and assay immediately or aliquot and store at ≤-20 °C. **Cell Lysates:**Cells must be lysed before assaying according to the following directions. 1. Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Resuspend cells in PBS (1×) and the cells was subject to ultrasonication for 4 times (or Freeze cells at ≤ -20 °C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.) 4. Centrifuge at 1500×g for 10 minutes at 2 - 8 °C to remove cellular debris. **Cell culture supernates and other biological fluids:** Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.
Sample Preparation:	• We are only responsible for the kit itself, but not for the samples consumed during the assay.

	The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance. • Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2). • If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. • Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals. • Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g.antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products. • Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit. • Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
Assay Procedure:	1. Prepare all reagents, samples and standards, 2. Add 100µL standard or sample to each well. Incubate 2 hours at 37 °C, 3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C, 4. Aspirate and wash 3 times, 5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C, 6. Aspirate and wash 5 times, 7. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C, 8. Add 50µL Stop Solution. Read at 450nm immediately.
Calculation of Results:	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 IL6 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. 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.

Application Details

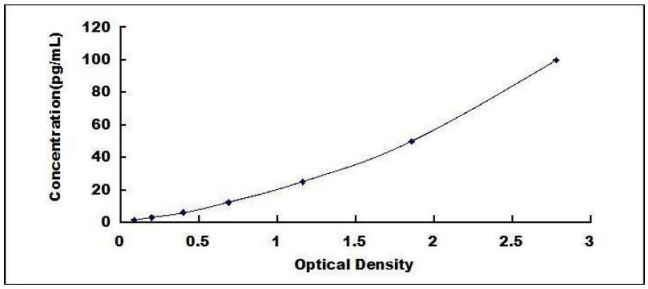
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level High Sensitive Interleukin 6 (IL6) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level High Sensitive Interleukin 6 (IL6) were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV<10% Inter-Assay: CV<12%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C
Storage Comment:	• For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt while the others should be at 4°C. • For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. • For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3 days at 37°C equaling 6 months at 4°C.
Expiry Date:	6 months

Publications

Product cited in:	Chiou, Jiang, Ding, Cheng: "Kidney-based in vivo model for drug-induced nephrotoxicity testing." in: **Scientific reports**, Vol. 10, Issue 1, pp. 13640, (2021) (PubMed).



ELISA

Image 1.