Datasheet for ABIN1028997

IL28B ELISA Kit

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Overview

Quantity:	96 tests
Target:	IL28B
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	15.625 pg/mL - 1000 pg/mL
Minimum Detection Limit:	15.625 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of IL28B in human 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 this index.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between this index and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between this index and all the analogues, therefore, cross reaction may still exist.
Sensitivity:	5.6 pg/mL

Product Details

Components:	• Pre-coated, ready to use 96-well strip plate • Standard (freeze dried) • Standard Diluent • Detection Reagent A • Detection Reagent B • Assay Diluent A • Assay Diluent B • TMB • Stop Solution • Wash Buffer (30X) • Plate sealer for 96 wells • Instruction manual
Material not included:	1. Microplate reader with 450 ± 10nm filter. 2. Precision single or multi-channel pipettes and disposable tips. 3. Eppendorf Tubes for diluting samples. 4. Deionized or distilled water. 5. Absorbent paper for blotting the microtiter plate. 6. Container for Wash Solution.

Target Details

Target:	IL28B
Alternative Name:	IL28B (IL28B Products)
Background:	Alternative name: IFNL3, Interferon,Lambda 3, Cytokine Zcyto22
Gene ID:	282617
UniProt:	Q8IZI9

Application Details

Sample Volume:	100 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	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 1 hour at 37°C

	6. Aspirate and wash 5 times 7. Add 90µL Substrate Solution. Incubate 15-25 minutes at 37°C 8. Add 50µL Stop Solution. Read at 450nm immediately.
Sample Collection:	Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2 °C - 8 °C within 30 minutes of collection. Store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20 °C After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at ≤ -20 °C. Cell culture supernates and Other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Note: 1. Samples to be used within 5 days may be stored at 2-8 °C , otherwise samples must stored at -20 °C (1 month) or -80 °C (2 months) to avoid loss of bioactivity and contamination. 2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals. 3. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit 4. Sample hemolysis will influence the result, so hemolytic specimen can not be detected. 5. When performing the assay slowly bring samples to room temperature. 6. Do not use heat-treated specimens.
Sample Preparation:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 °C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 °C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. 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.

1. Add 100 µl of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 °C.

2. Remove the liquid of each well, don't wash. Add 100 µl of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. and let it sit for 1~2 minutes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hour at 37 °C.

5. Repeat the aspiration/wash process for 5 times as conducted in step 3..

6. Add 90 µl of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 15-30 minutes at 37 °C. Protect from light.

7. Add 50 µl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Note:

1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.

2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards Detection Reagent A and B can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.

3. 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 have been added to the well strips, DO NOT let the strips DRY at any time during the assay.

4. For each step in the procedure, total dispensing time for addition of reagents to the assay

	plate should not exceed 10 minutes. 5. 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. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light. 9. The web version of manual is only for reference, subject to the instruction shipping with the kit.
Assay Procedure:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to the index. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific to the index. 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 the index, 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 the index in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. 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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-28B concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 1.3. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. Important note: 1. Limited by the current condition and scientific technology, we can't 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 2. 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. 3. Kits from different batches may be a little different in detection range, sensitivity and color developing time.Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website is only for information. 4. 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. 5. Do not remove microtiter plate from the storage bag until needed. 6. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement. 7. Use fresh disposable pipette tips for each transfer to avoid contamination. 8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. 9. 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. 10. 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. 11. Kits from different manufacturers for the same item might produce different results, since we haven't compared our products with other manufacturers. 12. The instruction manual also suit for the kit of 48T, but all reagents of 48T kit is reduced by half. 13. Valid period: six months.
Assay Precision:	• Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level the index were tested 20 times on one plate, respectively. • Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level the index were tested on 3 different plates, 8 replicates in each plate. • CV(%) = SD/meanX100 • Intra-assay: CV&lt10% • Inter-assay: CV&lt12%
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 ELISA 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 conditions. Note: To minimize unnecessary influences on the performance, operation procedures and lab conditions, especially room temperature, air humidity and incubator temperatures should be strictly regulated. It is also strongly suggested that the whole assay is performed by the same experimenter from the beginning to the end.
Storage:	4 °C,-20 °C
Storage Comment:	The Assay Plate, Standard, Detection Reagent A and Detection Reagent B should be stored at -20°C upon being received. After receiving the kit , Substrate should be always stored at 4°C.Other reagents are kept according to the labels on vials. But for long term storage, please keep the whole kit at -20°C. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
Expiry Date:	12 months