Version 25-04/25

User's Manual

Anti-Interferon beta Binding Antibodies ELISA Kit

REF **DEIA12535**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The kit is an enzyme immunoassay for in vitro quantitative measurement of Anti-IFNb in human serum, plasma and other biological fluids.

Principles of Testing

The microtiter plate provided in this kit has been pre-coated with IFNb. Standards or samples are then added tothe appropriate microtiter plate wells with a Horseradish Peroxidase (HRP)-conjugated secondary antibody. AfterTMB substrate solution is added, those wells that contain Anti-IFNb will exhibit a change in color. Theenzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change ismeasured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Anti-IFNb in thesamples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents And Materials Provided

Pre-coated, ready to use 96-well strip plate 1

Standard 2 vials

Standard Diluent 1×20mL

Detection Reagent A 1×120μL

Assay Diluent A 1×12mL

TMB Substrate 1×9mL

Stop Solution 1×6mL

Wash Buffer (30 × concentrate) 1×20mL

Plate sealer for 96 wells 4

Instruction manual 1

Materials Required But Not Supplied

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

Storage

1. For unused kit: The whole kit could be stored at -20°C in shelf life, while up to one month at 4°C. For experiment convenience, reagents could also be stored seperately, Standard, Detection Reagent A and 96-well strip plate should be stored at -20°C while the others could be at 4°C.
2. 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 the expiration date of the kit, please refer to the label on the kit box. All components are stable until this expiration date.

Specimen Collection And Preparation

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 1,000×g. Assay freshly prepared serumimmediately 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 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples inaliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Other biological fluids: Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

SAMPLE PREPARATION

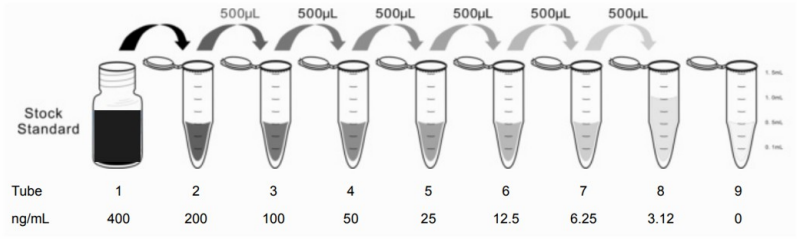
1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user shouldcalculate the possible amount of the samples used in the whole test. Please reserve sufficient samples inadvance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standardcurve, users must determine the optimal sample dilutions for their particular experiments. Sample should bediluted by PBS.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit isnecessary.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results dueto the impacts from certain chemicals.
5. 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

proteinsfrom other manufacturers may not be recognized by our products.

6. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernates may not be detected by the kit.
7. 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.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25oC) before use.
2. Standard: Reconstitute the Standard with 0.9mL 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 400ng/mL. Please firstly dilute the stock solution to 200ng/mL and the diluted standard serves as the highest standard (200ng/mL). Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to 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 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL,12.5ng/mL, 6.25ng/mL, 3.12ng/mL, and the last EP tubes with Standard Diluent is the blank as 0ng/mL.



3. Detection Reagent A: Briefly spin or centrifuge the stock Detection A before use. Dilute to the workingconcentration with working Assay Diluent A, respectively (1:100).
4. Wash Solution: Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled waterto 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. The standard for this kit is liquid. Due to its small volume, maybe invisible to the eye.
2. Making serial dilution in the wells directly is not permitted.
3. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37oC directly.
4. Please carefully reconstitute Standards or working Detection Reagent A according to the instruction, andavoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused bypipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than10μL for once pipetting.
5. The reconstituted Standards and Detection Reagent A can be used only once. 6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gentlyuntil the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.

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. Incubate for 1 hour at 37oC after coveringit with the Plate sealer.
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 remainingliquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 5 times. After thelast wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it againstabsorbent paper.
5. 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 SubstrateSolution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix theliquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensurethorough mixing.
7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on thesurface 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 frommicroplate. Rest wells should be resealed and stored at -20°C.
2. Samples or reagents addition:Please use the freshly prepared Standard. Please carefully add samplesto wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, totaldispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Thiswill ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards andspecimens, although not required, is recommended. To avoid cross-contamination, change pipette tipsbetween 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 isnecessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Oncereagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubationtime and temperature must be controlled.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for goodperformance. After the last wash, remove any remaining Wash Solution by aspirating or decanting andremove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poorprecision and false elevated absorbance reading.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observationonce every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strongreaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. Please protect it from light.

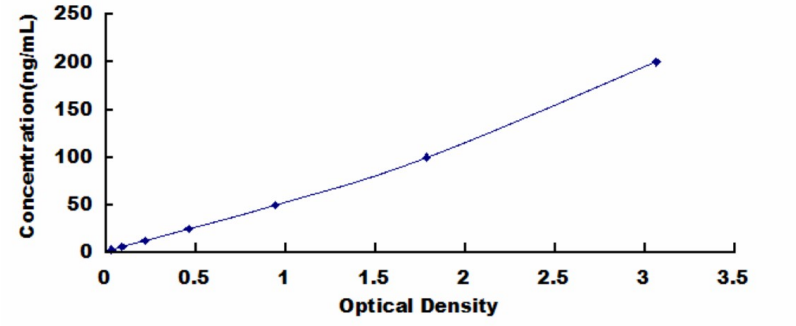
7. The environment humidity which is less than 60% might have some effects on the final performance,therefore, a humidifier is recommended to be used at that condition.

Calculation

Average the duplicate readings for each standard, control, and samples and subtract the average zero standardoptical density. Create a standard curve with Anti-IFNb concentration on the y-axis and absorbance on the x-axis.Draw a best fit curve through the points and it can be determined by regression analysis. If samples have beendiluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

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



Performance Characteristics

STABILITY

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 condition.

To minimize extra influence on the performance, operation procedures and lab conditions, especially roomtemperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that thewhole assay is performed by the same operator from the beginning to the end.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Anti-IFNb were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Anti-IFNb were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/mean×100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Detection Range

3.12-200ng/mL. The standard curve concentrations used for the ELISA's were 200ng/mL, 100ng/mL, 50ng/mL,25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL.

Sensitivity

The minimum detectable dose of Anti-IFNb is typically less than 1.19ng/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentrationthat could be differentiated from zero. It was determined by adding two standard deviations to the mean opticaldensity value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of Anti-IFNb. No significant cross-reactivity or interference between Anti-IFNb and analogues was observed.

Note:

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

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Anti-IFNb andtheir serial dilutions. The results were demonstrated by the percentage of calculated concentration to theexpected.

Sample	1：2	1：4	1：8	1：16
serum(n=5)	84-95%	90-105%	82-101%	91-99%
EDTA plasma(n=5)	97-104%	78-97%	89-103%	79-94%
heparin plasma(n=5)	81-97%	87-101%	85-98%	95-107%

Recovery

Matrices listed below were spiked with certain level of recombinant Anti-IFNb and the recovery rates werecalculated by comparing the measured value to the expected amount of Anti-IFNb in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	93-105	99
EDTA plasma(n=5)	80-94	85
heparin plasma(n=5)	90-101	95

Precautions

1. Limited by the current condition and scientific technology, we can't completely conduct the comprehensiveidentification and analysis on the raw material provided by suppliers. So there might be some qualitative andtechnical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, operation skills of the endusers 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 fromour website is only for information.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.5. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should becovered tightly to prevent the evaporation and contamination of microorganism.
6. There may be some foggy substance in the wells when the plate is opened at the first time. It will not haveany effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
7. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for theplate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and anoptical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbancemeasurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
8. Even the same operator might get different results in two separate experiments. In order to get betterreproducible results, the operation of every step in the assay should be controlled. Furthermore, apreliminary experiment before assay for each batch is recommended.
9. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with ourin-house data due to some unexpected transportation conditions or different lab equipments. Intra-assayvariance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers with the same item might produce different results, since we haven'tcompared our products with other manufacturers.
11. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.