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ABIN7014032

High Sensitivity Sandwich ELISA Kit for Interleukin 6

Human

96 tests

For research use only
Not for use in clinical diagnostic procedures
Version Aug 2022

Intended use

For quantitative detection of IL-6 in serum, plasma, tissue homogenates and other biological fluids.

Reagents and materials provided

- Pre-coated, ready to use 96-well strip plate
- Plate sealer for 96 wells
- Standard
- Sample/Standard Dilution Buffer
- Assay Diluent
- Biotin-labeled Antibody (Concentrated)
- HRP-Streptavidin (HRP-SA)
- Biotin System (BS)
- BS Dilution Buffer
- TMB Substrate
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

Materials required but not supplied

1. Microplate reader with 450 ± 10nm filter.
2. Precision single or multi-channel pipettes and disposable tips.
3. Microcentrifuge tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution
7. Automated plate washer
8. Incubator capable of maintaining 37 °C.

Storage of the kit

1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C.
2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.

Note:

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

Sample collection and storage

Sample type	Collection procedure
Plasma	Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8°C within 30 min of collection. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles. Hemolysed samples are not suitable for ELISA assay!
Serum	Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g. Collect the supernatant and assay freshly prepared serum 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 general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. Centrifuge the homogenates for 5 min at 5000×g to get the supernatant and assay immediately or aliquot and store at ≤-20°C.

Note:

1. 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.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent preparation

Bring all reagents and samples to room temperature for 20 minutes before use.

1. Wash Buffer: Dilute 30 mL Concentrated Wash Buffer to 750 mL Wash Buffer with deionized or distilled water. Put unused solution back at 2-8 °C.
   Note: 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 been dissolved. The solution should be cooled to room temperature before use.
2. Standards:
   a) Add 1 mL Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.
   Note: If the standard tube concentration higher than the range of the kit, please dilute it and label as zero tube.
   b) Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 mL of the Sample Dilution Buffer into each tube. Add 0.3 mL of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 mL from 1st tube to 2nd tube and mix them thoroughly, and so on. Sample Dilution Buffer is used for the blank control.
   Note: It is best to use Standard Solutions within 2 hours.
3. BS Working Solution: Prepare it within 15 minutes before experiment.
   a) Calculate required total volume of the working solution: 0.1 mL/well x quantity of wells. (Allow 0.1-0.2 mL more than the total volume.)
   b)Dilute the BS with BS Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 µL of BS into 99 µL of BS Dilution Buffer.)
   Note: If crystals have formed in the BS, you can warm it with water (temperature should not exceed 30 °C) and mix it gently until the crystals have completely been dissolved.

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior

to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.

- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Assay procedure

1. Set standard, test samples, control (blank) wells on the pre-coated plate respectively.
2. Add 50μL of Assay Diluent to each well.
3. Add 50μL each of the dilutions of standard (see Reagent Preparation), blank and samples to the appropriate wells.
4. Seal the plate with a cover and incubate at 37°C for 90 minutes.
5. Remove the cover and discard the plate content, wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
6. Add 100μL Biotin-labeled antibody to each well. Cover the plate and incubate at 37°C for 60 minutes.
7. Remove the cover and wash plate 2 times with Wash Buffer, let the Wash Buffer stay in the wells for 1-2 minutes each time.
8. Add 100μL of HRP-SA to each well, cover the plate and incubate at 37°C for 30 minutes.
9. Remove the cover and wash plate 3 times with Wash Buffer, let the Wash Buffer stay in the wells for 1-2 minutes each time.
10. Add 100μL of BS Working Solution to each well. Cover the plate and incubate at room temperature for 15 minutes.
11. Remove the cover and wash plate 3 times with Wash Buffer.
12. Add 100μL of HRP-SA to each well, cover the plate and incubate at 37°C for 30 minutes.
13. Remove the cover and wash plate 3 times with Wash Buffer, let the Wash Buffer stay in the wells for 1-2 minutes each time.

14. Add 90μL TMB Substrate to each well, cover the plate and incubate at 37°C in the dark within 10-20 minutes. (Note: The reaction time can be shortened or lengthened depending on the actual color change, but must not exceed 30 minutes. The reaction can be stopped when a visible color gradient appears in the standard wells.)
15. Add 50μL Stop Solution to each well. The color will turn yellow immediately. The Stop Solution should be added in the same order as the TMB Substrate.
16. Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the Stop Solution.

Test principle

The kit is based on sandwich enzyme-linked immunosorbent assay technology. The capture antibody is pre-coated on the 96-well plate and a biotin-conjugated antibody is used as the detection antibody. The standards, test samples and biotin-conjugated detection antibody are added sequentially to the wells and washed with wash buffer. HRP-Streptavidin (HRP-SA) and Biotin System (BS) are added and unbound conjugates are washed off with wash buffer. Then, by dual signal amplification, TMB substrates are used to visualize the enzymatic HRP reaction. TMB is catalyzed by HRP to produce a blue product that turned yellow after the addition of acidic stop solution. The density of yellow color is proportional to the target amount of samples. Read the absorbance at 450 nm in a microplate reader to calculate the concentration of the target product.

Calculation of results

Average the duplicate readings for each standard, control, and samples and subtract the average of the measured optical density of the zero standard. Create a standard curve by plotting the mean OD value 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 concentration on the y-axis and absorbance on the x-axis. The use of plotting software is also recommended. If the 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 log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. 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).

Detection range

The detection range of the kit is 0.078 pg/mL - 5 pg/mL

Sensitivity

The minimum detectable dose of IL6 is typically less than 0.046 pg/mL.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.
It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Note:
Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between IL6 and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-Assay: CV<8% Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to 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 monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Assay procedure summary

1. Prepare all reagents, samples and standards,
2. Add 50µL Assay Diluent to each well
3. Add 50µL standard or sample to each well. Incubate 90 minutes at 37 °C,
4. Aspirate and wash 2 times,
5. Add 100µL Biotin-labeled antibody to each well. Incubate 1 hour at 37 °C,
6. Aspirate and wash 2 times,
7. Add 100µL BS Working Solution to each well. Incubate 15 minutes at RT,

8. Aspirate and wash 3 times,
9. Add 100μL HRP-SA to each well. Incubate 30 minutes at 37 °C,
10. Aspirate and wash 3 times,
11. Add 90μL Substrate Solution. Incubate 10-20 minutes at 37 °C,
12. Add 50μL Stop Solution. Read at 450nm immediately.

Important note

1. 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.
2. Limited by the current conditions and scientific technology, we cannot perform a complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.
3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.
9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and

does not affect the test results. Return unused wells to the foil pouch and store as before.

11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of 450 ± 10 nm is suitable for absorbance measurement. Please read the instructions carefully and set up the instrument before the experiment.
12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Troubleshooting

Problem	Possible Source	Corrective Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature

Problem	Possible Source	Corrective Action
	Conjugate or substrate reagent failure	Mix conjugate and substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Resample and repeat assay

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