

Human IL-6 ELISA KIT

Cat #: orb1085834 (manual)

Size: 96 tests

This kit is used to quantify the amount of interleukin-6 (IL-6) in samples such as human serum, plasma, or cell culture supernatants. Please read the manual carefully and check the reagent components before use. If you have any questions, please contact biorbyt, we will provide you with technical support.

For research use only, not for clinical diagnosis.

Detection Principle

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Purified interleukin-6 (IL-6) antibody was coated in the microplates, then the standard or the samples to be tested are added to the coated plate wells. After the antigen fully reacts with the coated antibody, the biotinylated IL-6 antibody and streptavidin labeled with HRP are added in turn. The biotinylated IL-6 antibody and streptavidin form high-intensity non-covalent binding. After sufficient washing, the substrate TMB is added for color development. TMB is converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The color shading is proportional to the IL-6 content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of IL-6 in the sample was calculated by plotting the standard curve.

Product Composition

Reagents	Specifications (96T)	Storage Conditions
Antibody-Coated Slats	8×12	2-8°C
Standard	2 tubes	2-8°C
S1 standard/sample diluent	16 ml ×1 bottle	2-8°C
Concentrated Biotin-Labeled Antibody (100×)	60 µl ×2 bottles	2-8°C
S2 Biotin-Labeled Antibody diluent	16 ml ×1 bottle	2-8°C
Concentrated HRP-Streptavidin Conjugate (100×)	60 µl ×2 bottles	2-8°C
S3 enzyme conjugate diluent	16 ml ×1 bottle	2-8°C
Concentrated Washing Buffer (20×)	25 ml×1 bottle	2-8°C
Chromogenic substrate (protect from light)	12 ml×1 bottle	2-8°C
Stop Solution	12 ml×1 bottle	2-8°C
Plate Sealer	4 pieces	
Manual	1 copy	

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision single (0.5-10 μ L, 2-20 μ L, 20-200 μ L, 200-1000 μ L) and multi-channel pipette with disposable tips (calibration is required before use.)
3. Automated plate washer
4. 37°C incubator
5. Deionized or distilled water
6. Coordinate paper
7. Measuring cylinder

Precautions

1. The kit is stored in 2-8°C and the dissolved but unused standard is recommended for disposal. Do not mix kit components from different sources or batch numbers, use this product within the expiration date.
2. When the concentrated washing solution is taken out at low temperature, there may be crystal precipitation, and it can be dissolved by heating in the water bath before dilution, which does not affect the use.
3. Pipettes should be calibrated before and used for each step and to avoid errors. It is recommended to control the sampling time within 5 minutes. If there are a large number of samples, it is recommended to use a multi-channel pipette for sampling.
4. Please make a standard curve at the same time of each determination, and it is better to make a double-check well. If the content of the substance to be tested in the sample is higher than the upper limit of the reagent kit (the OD value of the sample is greater than the OD value of the first well of the standard well), please dilute a certain multiple with the sample dilution buffer before determination. The total dilution multiple shall be multiplied during calculation.
5. In order to avoid cross contamination, remember to replace the tip when adding different concentrations of standard, different samples and different reagents. The plate sealer is only for single use.
6. The concentrated HRP conjugate and TMB substrate should be protected from light. The TMB substrate should be kept colorless before adding. Do not use the TMB substrate when it turned blue.
7. Strictly follow the manual, and the test results must be based on the microplate reader reading.

Sample Collection and Storage

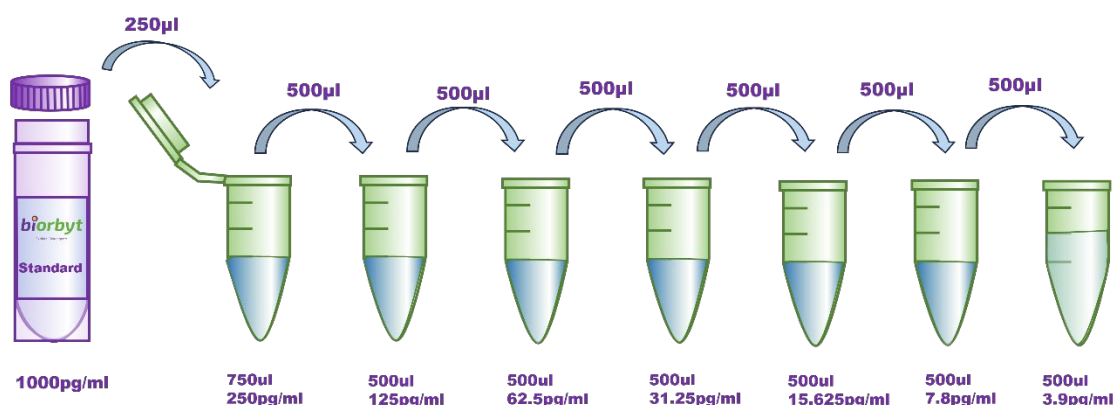
1. **Serum:** Blood coagulated naturally at room temperature for 30 min and centrifuged for 20 min (2000-3000 rpm). Collect supernatant carefully. If precipitation occurs during storage, centrifuge again to avoid repeated freezing and thawing.

- Plasma:** Select EDTA or citric acid as anticoagulant according to sample requirements and centrifuge for about 20 min (2000-3000 rpm). Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.
- Cell supernatant:** When detecting secretive components, collect with a sterile tube. Centrifuge for about 20 min (2000-3000 rpm). Collect the supernatant carefully.
- If the sample cannot be tested immediately, dispense it according to the minimum amount of use, and store it in -20°C-70°C to avoid repeated freezing and thawing. Avoid hemolytic or hyperlipidemia samples. If the serum contains a large amount of particles, centrifuge or filter to remove them before testing; Thaw at room temperature, do not heat thaw at 37°C or higher.
- Samples containing NaN₃ could not be tested because NaN₃ inhibited horseradish peroxidase activity.
- Please dilute the sample in appropriate fold according to the actual situation (it is recommended to determine the dilution fold according to the pre-test results).

Note: It is recommended to dilute serum or plasma samples at least 1:2.

Reagent Preparation

- Reagent reheating:** Please reheat the reagent kit and the sample to be tested at room temperature within 30 minutes before the test.
- Preparation of Washing Buffer:** According to the concentration ratio of the concentrated washing buffer, dilute it with double distilled water or deionized water at the corresponding ratio for later use.
- Standard gradient dilution:** Take 1ml of standard/sample diluent (S1) into the freeze-dried standard, let it stand for 15 minutes until it is completely dissolved, then gently mix (concentration of 1000pg/ml), and then take 7 polypropylene test tubes. Add 750 μ l of standard/sample diluent (S1) to the first test tube, and 500 μ l of standard/sample diluent (S1) to the other tubes. Dilute twice according to the following concentrations: 250, 125, 62.5, 31.25, 15.625, 7.8, 3.9pg/ml. 250pg/ml is the highest concentration point on the standard curve, and the standard/sample diluent (S1) is used as the zero point (0pg/ml) on the standard curve. The standard stock solution (1000pg/ml) that has not been used up after re dissolving should be discarded or packaged according to a single dosage as needed, and stored in a refrigerator at -20~-80 °C.



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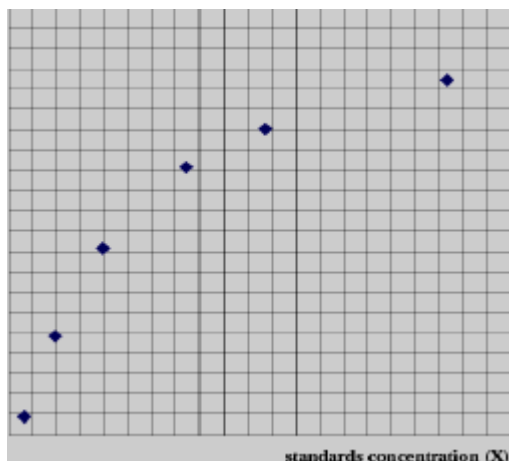
4. Biotinylated antibody working solution: According to the required amount for the experiment, dilute the Concentrated Biotin-Labeled Antibody (100×) with Biotin-Labeled Antibody diluent (S2) to 1×. Please use within 30 minutes.
5. Enzyme conjugate working solution: According to the required amount for the experiment, dilute the Concentrated HRP-Streptavidin Conjugate (100×) with enzyme conjugate diluent (S3) to 1×. Please use within 30 minutes.

Operation Steps

1. Sample adding: Take out the Antibody-Coated Slats according to the required amount of the test, respectively add 100μl of the prepared standards, standard zero point and the samples to be tested to the bottom of the wells.
2. Incubation: Seal the plate with Plate Sealer and let it incubate at 37 °C for 90 minutes (excluding blank control wells).
3. Washing: Carefully remove the Plate Sealer, discard the liquid, shake it dry, fill each well with washing solution (350 μl), let it stand for 30 seconds, then discard it. Repeat this process 4 times, and finally pat dry on absorbent paper.
4. Biotin-Labeled Antibody adding: Add 100μl of Biotin-Labeled Antibody to each well and mix thoroughly (excluding blank control wells).
5. Incubation: Seal the plate with the Plate Sealer, incubate for 60 min at 37°C.
6. Washing: The same washing process as above (step 3), wash the plate 4 times.
7. HRP-Streptavidin Conjugate adding: Add 100μl HRP-Streptavidin Conjugate to each well and mix thoroughly (excluding blank control wells).
8. Incubation: Seal the plate with the Plate Sealer, incubate for 30 min at 37°C.
9. Washing: Same as the above washing process (Step 3), wash the plate for 4 times.
10. Color development: Add 100 μl of Chromogenic substrate to each well, seal the plate with Plate Sealer, and let it develop color at 37 °C for 10-20 minutes.
11. Termination: Add 100μl of Stop Solution to each well (at this time, blue turns to yellow).
12. Determination: Measure the absorbance (OD value) of each well at the microplate reader with a wavelength of 450nm. The measurement should be performed within 5 min after adding the stop solution.

Result Judgment

1. The OD value of each standard and sample minus the OD value of the blank well is the final value, and if a duplicate well is made, its mean value shall be calculated.
2. Take the absorbance OD value as the ordinate (Y) and the corresponding standard concentration as the abscissa (X) to generate the corresponding standard curve. The IL-6 content of the sample can be calculated from the standard curve according to its OD value. If the OD value of the sample is higher than the upper limit of the standard curve, perform the appropriate dilution during the test, and then multiply the concentration of the sample by the corresponding dilution.



This drawing is for reference only and shall be based on the standard curve drawn in the actual test

Kit Performance

The difference between batches should be less than 10%

Detection Range

3.9 pg/ml -250 pg/ml

Sensitivity

2 pg/ml

Troubleshooting

Problems	Possible Causes	Solutions
No signal	Mixing Reagents with Different ELISA kits or Batch Numbers	Recheck the label of the reagents to make sure that all components are in the testing kit being used. Do not mix reagents of different testing kits or batch numbers.
	Missing antibody, enzyme and chromogenic agent	Check the operation procedure, and be careful not to omit adding.
	HRP enzyme contaminated with sodium azide	Re-preparation of reagent, no sodium azide
	Wrong reagent preparation/use	Redo the test, operate in strict accordance with the manual, and see the labels clearly before each preparation and use
Weak signal	Reagents Expires expiration date	Check product validity
	Insufficient incubation time	Check the incubation time
	Use of contaminated reagents	Check if reagent is contaminated
	Incorrect instrument setting, filter mismatch	Whether the instrument is set correctly and the filter is used, etc.
	Washing operation is not standard	If the washing is insufficient, increase the number of washing times or extend the washing time
		Wash the bottle, each well shall be completely filled with washing buffer, and pour out quickly
		If a plate washer is used, it shall be calibrated and set to a volume sufficient to fill each hole and the inside of the plate shall not touch the equipment
		Check whether there is residual washing liquid in each well or the volume of sample added in each well is accurate
		You can add a 30 second soak between washings

High background	Improper incubation temperature and time in the experiment	Determine the appropriate incubation temperature and time for each test step
	Excessive enzyme addition	Check whether the regulating amount of pipette is correct before adding enzyme
		Check dilution and perform titer determination if necessary
The standard curve is good, but the sample wells have no signal	Low content of target in sample or no target in sample	Set the positive control and repeat the experiment
	Sample matrix effect influence detection	Test again after re-diluting sample
The standard curve is good, but the sample wells have high signal	The content of sample to be tested exceeds the standard curve range	Test again after re-diluting sample