Human IL-6 ELISA Kit

Catalog Number: I60111A-96

【INTENDED USE】

This kit is suitable for the quantitative determination of Human IL-6 concentration in serum and cell culture supernatant. Due to the complexity and unpredictable interference of natural samples, it is recommended that users explore the best detection conditions for natural samples with the kit.

【PRINCIPLE】

This kit using double antibody sandwich enzyme-linked immunosorbent detection technology to determinate residues of Human IL-6 in the samples. The capture antibody is coated with 96-well labeling plate and the solid phase antibody is prepared. Then first add the standard and testing samples, and second put a marker on horseradish peroxidase labeled antibody, forming a solid phase antibody-Human IL-6- labeled antibody sandwich, reaction after washing, then add the chromogenic reaction of the substrate. The substrate under the catalysis of HRP become blue, and under the action of stop solution into the final yellow. Determine its absorbance value (OD value) at wavelengths of 450 nm and 630 nm, where, 630 nm is the corrected wavelength. The OD value is positively correlated to content of Human IL-6 in the sample .

【MATERIALS PROVIDED】

Number	Component	Size	Storage condition
1	Microtiter Plate	96T	2-8℃
2	Standard(3200 pg/mL)	1 mL×1 tube	2-8℃
3	HRP-Conjugate Antibody	10 mL×1 vial	2-8℃
4	Sample Dilution	30 mL×1 vial	2-8℃
5	Wash Buffer (20×)	30 mL×1 vial	2-8℃
6	Developing solution A	8 mL×1 vial	2-8℃ (keep in dark)
7	Developing solution B	8 mL×1 vial	2-8℃ (keep in dark)
8	Stop Solution	15 mL×1 vial	2-8℃
9	Microplate Sealers	3	-

【STORAGE】

The kit should be stored away from light at 2-8 ℃ for 12 months. The unused kits should be kept away from light at 2-8 ℃ after opening. The production date and expiration date can be found on the kit label.

【REAGENTS/EQUIPMENTS REQUIRED BUT NOT SUPPLIED】

1. Enzyme marker, constant temperature oscillator (or constant temperature incubator), washing machine, vortex oscillator, timer;
2. High-precision pipettes and disposable suction heads (0.5-10 μL, 10-100 μL, 30-300 μL, 100-1000 μL);
3. Deionized water (used to prepare wash buffer (1×));
4. Absorbent paper, EP tube, disposable gloves.

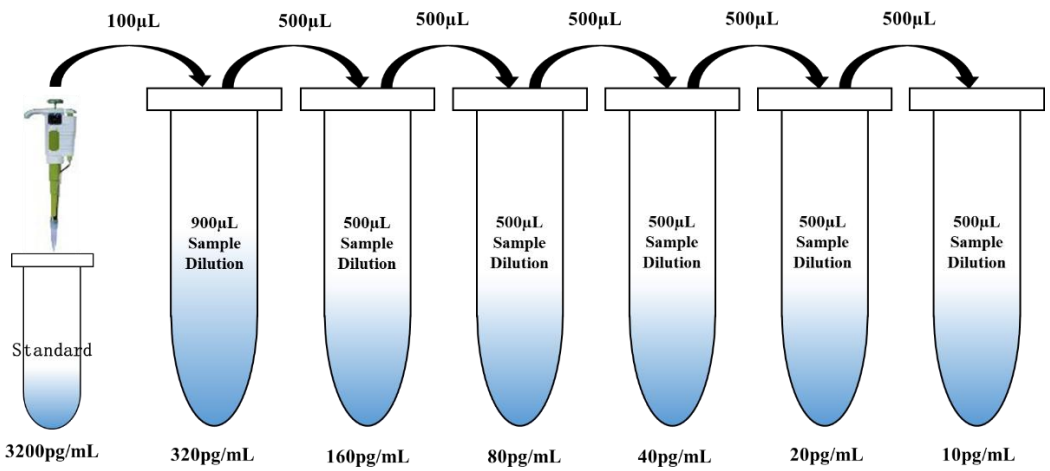
【REAGENT PREPARATION】

- Wash buffer (1×) Preparation Take 1 part of wash buffer (20×) and add 19 parts of deionized water to prepare wash buffer (1×). If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- Preparation of substrate liquid Mix the color developing liquid A and B in the same volume, and place away from light after mixing. (Note: the time cannot be placed for too long, generally used before 10min preparation. Do not use if the mixture has turned blue).
- Preparation of the standard product Dilute the standard product to 320pg/mL with the sample diluent, and then prepare the standard product with the method of double dilution (the newly prepared standard solution is used for each experiment).

【 ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃ .
2. Preparation of the standard curve: dilute the Standard with Sample dilution to 320 pg/mL, then prepared by Multiple Dilution method.



3. Sample warming: 50μL of standard substance/blank (sample diluent)/sample was added to each well first, then 50μL of HRP-Conjugate Antibody was added to each well, and the plate was sealed with a sealing plate membrane, and then incubated at 37℃ and 500rpm for 1h under shock. (Note: If the plate is not sealed or the plate is not completely sealed during warming, the reaction liquid will evaporate, resulting in error in the experiment)
4. Wash the plate: After warming, carefully remove the sealing plate film, discard the liquid in the well, wash the plate with wash buffer (1×) for 3 times (250μL/ well), and pat the residual liquid in the sample well dry. (Note: If the hand washing board is used, the washing liquid (1×) needs to be suspended when adding liquid, and the gun head is best not to touch the inner wall of the well; After each addition of wash buffer (1×), let it stand for 30s and shake slightly; When patting dry, pay attention to changing the absorbent paper or patting the clean area on the paper each time.)
5. Color development: Add the pre-configured substrate liquid to the enzymic label plate at 100μL/ well, seal the plate with a sealing plate film, and let it stand at 37℃ for 15min (do not shake) away from light.
6. Termination: Add the termination liquid, 100μL/ well, and the reading can be done after the color is uniform.
7. Reading: Put the label plate into the label meter, set the wavelength to dual wavelength 450/630nm, read the absorbance value, and the determination should be completed within 20min after termination. (Note: It is recommended to set the shock of 5-10s in the reading program of the enzyme marker)

【 CALCULATION OF RESULTS】

1. Calculation of absorbance value: The calibrated value of light absorbance of each standard or sample is: ($OD_{450\ nm}$-$OD_{630\ nm}$)- absorbance valueof the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$).
2. Plot the standard curve with concentration of the standard as the horizontal coordinate (X) and the calibrated value of light absorption of the standard as the vertical coordinate (Y), and a four-parameter Logistic mathematical model is recommended to fit the equation:Y=（（A-D）/（1+（X/C）^B））+D.Substitute OD value of the sample ($OD_{450\ nm}$-$OD_{630\ nm}$) - absorbance value of the blank control well($OD_{450\ nm}$-$OD_{630\ nm}$)) into the formula to calculate the content of Human IL-6 in the sample, and note the dilution factor.
3. If the OD value of the sample to be tested exceeds the highest OD value of the standard curve, the sample shall be redetermined after dilution.

【PRECAUTIONS】

1. All components in the kit must be restored to room temperature (20-25℃) before use.

2. All components are thoroughly mixed before use, and the standard product needs to be centrifuged briefly for 5 seconds to concentrate the liquid on the tube wall and lid at the bottom of the tube; Return all reagents to 2-8℃ immediately after use.
3. The kit must be used within the validity period, and the corresponding standard curve must be prepared for each test. It is not recommended to use different batches of related reagents in mixed batches.
4. When patting dry after washing, pay attention to prevent the slats from falling off.
5. Only in strict compliance with the operation methods of the instructions, all the reagents of this kit are used, and the sampling tank and suction head are replaced in time between different samples and steps to avoid cross-contamination.
6. The final test results are closely related to the effectiveness of the reagent, the operator's operation method and the test environment.
7. The company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible use of the sample before use, and reserve sufficient samples.
8. This kit is for research use only, not for clinical diagnosis.

【SAFETY INSTRUCTIONS】

1. All biological samples have potential biosafety risks, and users should handle and discard samples in strict accordance with local laws and relevant regulations.
2. The operator should wear personal protective equipment, such as lab coat, gloves, mask and goggles.

【PROBLEM ANALYSIS】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well
The color development is weak or	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature

colorless	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly
		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use

	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution
	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use

The specification is issued in version A/0 with an effective date of 28th Oct,2024.

Name：Shanxi Junyan Bioscitech Co., Ltd
Address：No.96,Tanghuai St. Xiaodian Dist., Taiyuan, Shanxi, P.R. China
Net：www.junyanbios.cn
Order：15234188795
Support：19503412257
Email：sales@junyanbios.cn