Rat IL5 ELISA Kit

Cat #: orb1809034 (manual)

Size: 96 tests

This kit is used to quantify the amount of interleukin-5 (IL-5) in samples such as rat 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-5 (IL-5) 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-5 antibody and streptavidin labeled with HRP are added in turn. The biotinytin 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-5 content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of IL-5 in the sample was calculated by plotting the standard curve.

Product Composition

Reagents	Specifications（96T）	Storage Conditions
Antibody-Coated Slats	8×12	-20℃
Standard	2 bottles	-20℃
Dilution Buffer	45 ml×1 bottle	-20℃
Detection Solution A	120 µl×1 tube	-20℃
Detection Solution B	120 µl×1 tube	-20℃
Washing Buffer（Concentrated,30×）	20ml×1 bottle	2-8℃

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

TMB Substrate（Avoid direct light）	9 ml×1 bottle	2-8℃
Stop Solution	6 ml×1 bottle	2-8℃
Plate Sealer	4 pieces	
Manual	1 copy	

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)

2. Precision single (0.5-10μL, 2-20μL, 20-200μL, 200-1000μL) 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 4℃& -20℃, 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 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 60-120 min and centrifuged for 20 min (1000 rpm). Collect supernatant carefully. If precipitation occurs during storage, centrifuge again to avoid repeated freezing and thawing.

2. **Plasma**: Select EDTA or citric acid as anticoagulant according to sample requirements and centrifuge for about 20 min (1000 rpm). Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.

3. **Cell supernatants or other biological samples**: When detecting secretive components, collect with a sterile tube. Centrifuge for about 20 min (1000 rpm). Collect the supernatant carefully.

4. Tissue homogenization: 1) Take an appropriate amount of tissue mass, wash it in pre-cooled PBS to remove blood, and use it after weighing (if the tissue mass is large, it needs to be shredded first and then homogenized); 2) A variety of homogenization methods can be used at the same time to achieve better crushing effect: First, the tissue mass is moved into a glass homogenizer, and 5-10 mL of pre-cooled PBS is added for full grinding. The process needs to be carried out on ice; the obtained homogenate can be further processed by ultrasonic crushing or repeated freezing and thawing; 3) Centrifuge the prepared homogenate at 5000 × g for 5 minutes and remove the supernatant.

5. Cell lysate: 1) Adherent cells need to be digested with pancreatic enzymes first, collected by centrifugation, and the cells (suspended cells can be collected directly by centrifugation); 2) Wash the collected cells with cold PBS for 3 times; 3) Lyse the cells by physical methods (the cells can be broken by ultrasound first, and then frozen and thawed repeatedly); 4) Centrifuge the specimen at 4 ° C 1500 × g for 10 minutes, collect the supernatant and set aside.

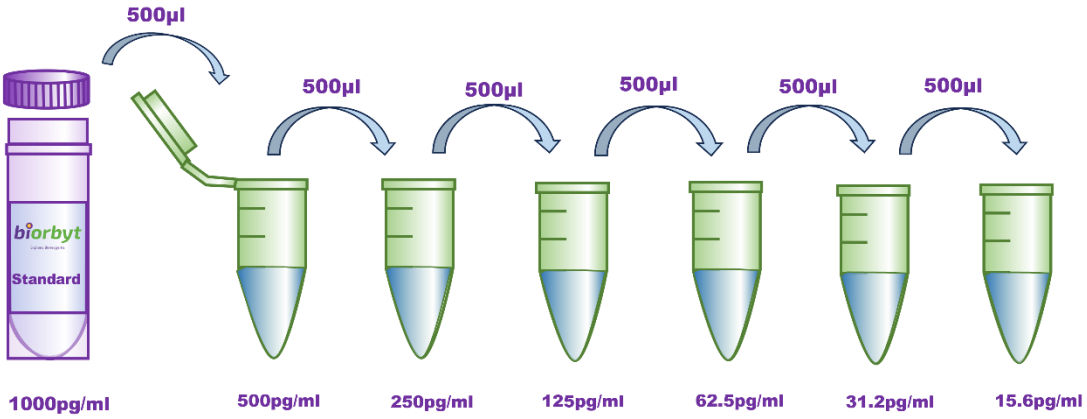
6. If the sample cannot be detected immediately, please pack it in the smallest amount and store it at -20 ° C to 70 ° C to avoid repeated freezing and thawing. Try to avoid using hemolytic or hyperlipidemic samples.

Reagent Preparation

1. Reagent reheating: Please reheat the reagent kit and the sample to be tested at room temperature within 30 minutes before the test.

2. Preparation of Washing Buffer: Dilute the concentrated Washing Buffer (30×) to Washing Buffer working solution (1×) with double distilled water or deionized water, and keep it as standby.

3. Gradient dilution of standard: Take 1 ml of Standard/Sample Dilution Buffer into the lyophilized standard, allow it to stand for 15 min until it is completely dissolved, then gently mix it with a concentration of 1000pg/ml, take 6 EP tubes, add the tubes with 500µl of Standard/Sample Dilution Buffer, and dilute twice according to the following concentration: 500, 250, 125, 62.5, 31.2, 15.6pg/ml were diluted. 1000pg/ml is the highest concentration of the standard curve, and the Standard/Sample Dilution Buffer is the zero point (0pg/ml) of the standard curve. The Standard Stock Solutions (1000pg/ml) that has not been used up should be discarded.



4. Detection Solution A and Detection Solution B: Before use, please shake a few times or less centrifugal treatment, so that the liquid of the tube wall or cap is deposited to the bottom of the tube. Before use, dilute the diluent 1:100 (eg: 10 µL detection solution A/990 µL dilution), mix well, prepare according to the pre-calculated total amount required for each experiment (100 µL/well) before dilution, and prepare more 0.1-0 mL in actual preparation.

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 the Plate Sealer, incubate for 120 min at 37℃.

3. Discard the liquid, shake dry, no need to wash.

4. Add detection solution A working solution (prepared before use): add 100 µl of detection solution A working solution to each well.

5. Incubation: Seal the plate with the Plate Sealer and incubate at 37 ° C for 60 min.

6. Washing: Carefully remove the Plate Sealer, discard the liquid, shake dry, fill each well with washing buffer (350 µl), let stand for 1-2 minutes and discard, repeat 3 times, finally pat dry on absorbent paper.

7. Add detection solution B working solution (prepared before use): add 100 µl of detection solution B working solution to each well.

8. Incubation: Seal the plate with the Plate Sealer and incubate at 37 ° C for 60 min.

9. Washing: Wash the plate 5 times as washing process before (step 5).

10. Color development: Add 90 µl of color-developing TMB substrate solution to each well, and seal the plate with the Plate Sealer at 37 ° C for 15-25 minutes.

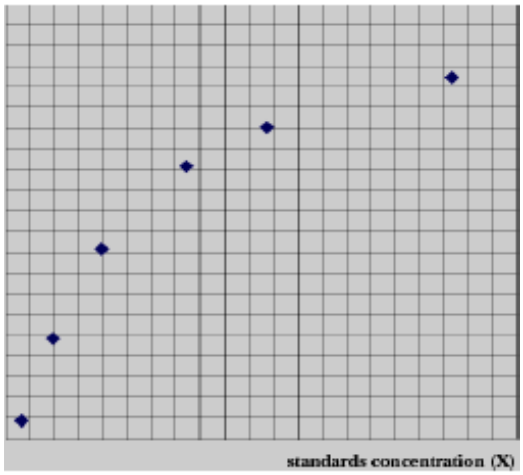
11. Termination: Add 50 µl of stop solution to each well (at this time, the blue immediately turns yellow).

12. Determination: Measure the absorbance (OD value) of each well at a wavelength of 450 nm with a microplate reader. The measurement should be carried out within 5 minutes after adding the stop solution.

Result Judgment

1. The OD value of each standard and sample minus the OD value of the zero 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-5 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

15.6 pg/ml -1000 pg/ml

Sensitivity

6.4 pg/ml