Mouse Osteocalcin (OC) ELISA Kit

Cat #: orb1974583 (manual)

Size: 96 tests

This kit is used to quantify the amount of Osteocalcin (OC) in samples such as mouse serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids. 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 osteocalcin (OC) 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 osteocalcin 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 osteocalcin content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of osteocalcin 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 tubes	-20℃
S1 Standard/Sample Dilution Buffer	45 ml×1 bottle	-20℃
Detection Solution A	120µl×1	-20℃
Detection Solution B	120µl×1	-20℃
Washing Buffer (Concentrated, 30×)	20ml×1	2-8℃
TMB Substrate(Avoid direct light)	9 ml×1	2-8℃
Stop Solution	6 ml×1	2-8℃
Plate Sealer	4 pieces	
Manual	1 copy	

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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 2-8℃& -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.

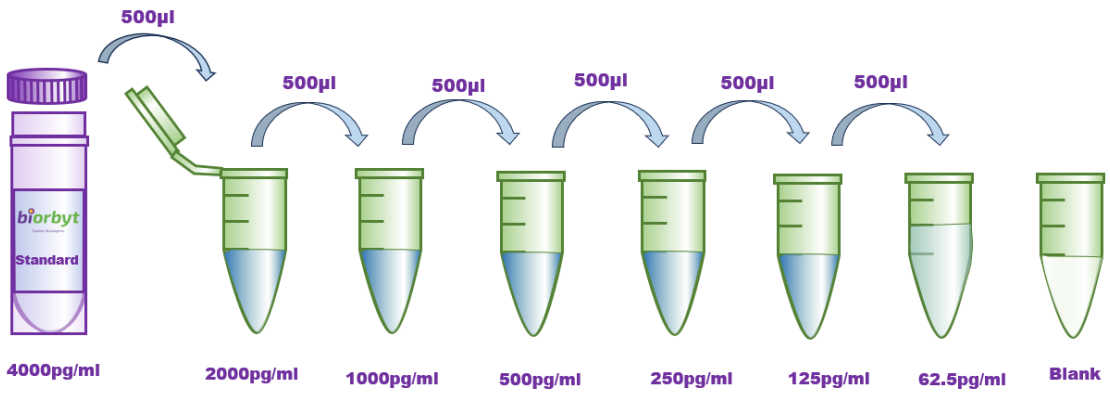
Sample Collection and Storage

1. **Serum**: Blood coagulated naturally at room temperature for 60-120 min and centrifuged for 20 min at 1000g. 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 at 1000g. Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.

3. **Cell supernatant**: When detecting secretive components, collect with a sterile tube. Centrifuge for about 20 min at 1000g. Collect the supernatant carefully.
4. **Tissue homogenization**: 1) Take an appropriate amount of tissue blocks, wash them in pre-cooled PBS to remove blood, weigh them and set aside them (larger tissue blocks need to be cut and then homogenized); 2) A variety of homogenization methods can be used at the same time to achieve a better crushing effect: first, the tissue block is moved into the glass homogenizer, and 5-10mL of pre-cooled PBS is added for full grinding, and the process needs to be carried out on ice; The obtained homogenate can be reused for further treatment by ultrasonic crushing or repeated freeze-thawing; 3) Centrifuge the prepared homogenate at 5000×g for 5 minutes, and take the supernatant.
5. Cell lysate: 1) Adherent cells need to be trypsinized and centrifuged to collect cells (suspension cells can be collected by centrifugation); 2) Wash the collected cells 3 times with cold PBS; 3) Physical method to lyse cells (cells can be broken by ultrasonication first, and then repeated freeze-thaw); 4) Centrifuge the specimen at 4°C 1500×g for 10 min and collect the supernatant for later use.
6. If the sample cannot be tested immediately, dispense it according to the minimum amount of use, and store it in -20℃-70℃ to avoid repeated freezing and thawing. Avoid hemolytic or hyperlipidemia samples.

Reagent Preparation

1. Reagent reheating: Please reheat the reagent kit and the sample to be tested at room temperature 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 (S1) into the lyophilized standard, allow it to stand for 15 min until it is completely dissolved, then gently mix it with a concentration of 4000pg/ml, take 6 EP tubes, add 500µl of each dilution and dilute 2-fold at the following concentrations: 2000, 1000, 500, 250, 125, 62.5pg/ml. 4000 pg/ml is the highest point concentration of the standard curve, and the dilution is used as the zero point of the standard curve (0 pg/ml). The reconstituted standard stock solution (4000 pg/ml) should be discarded if it is not used up.



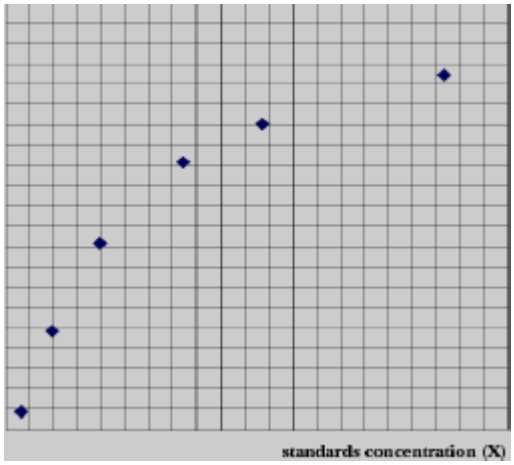
4. Detection solution A and detection solution B: Centrifuge with a few shakes or less before use to allow the liquid from the tube wall or bottle cap to be deposited to the bottom of the tube. Before use, dilute 1:100 with dilution (e.g., 10 μL detection solution A/990 μL dilution), mix well, prepare according to the total amount required for each experiment (100 μL/well) before dilution, and prepare 0.1-0.2mL more when actually pre-comparing.

Operation Steps

1. Loading: According to the amount required for the test, take out the corresponding antibody-coated strips, and add the prepared standard, standard zero point and the sample to be tested to the bottom of the experimental well at 100μl/well.
2. Incubation: Seal the plate with sealing tape and then incubate at 37°C for 120min.
3. Discard the liquid, spin dry, and do not wash.
4. Add detection solution A working solution (prepared before use): Add 100μl of detection solution A working solution to each well.
5. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 min.
6. Washing: Carefully remove the sealing tape, discard the liquid, spin dry, fill each well with washing solution (350μl), and let it stand for 1-2min, discard, repeat 3 times, and finally pat dry on absorbent paper.
7. Add detection solution B working solution (prepared before use): Add 100μl of detection solution B working solution to each well.
8. Incubation: Seal the plate with adhesive tape and incubate at 37°C for 60 min.
9. Washing: Wash the plate 5 times as described above (step 5).
10. Color development: 90 μl of chromogenic substrate solution was added to each well, and the plate was sealed with sealing tape and then left at 37°C for color development for 15-25min.
11. Termination: Add 50 μl of terminator solution per well (blue to yellow at this point).
12. Determination: The absorbance (OD value) of each well was determined with a microplate reader at a wavelength of 450nm, and the determination should be done after adding the stop solution proceed within 5 minutes.

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 osteocalcin 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

62.5 pg/ml - 4000 pg/ml

Sensitivity

23.58 pg/ml

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