Mouse FGF6 ELISA Kit

Cat #: orb1809233 (manual)

Size: 96 tests

For research use only, not for clinical diagnosis.

This kit is used to quantify the amount of FGF6 in samples such as mouse serum, plasma 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.

Detection Principle

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Purified FGF6 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 FGF6 antibody and streptavidin labelled with HRP are added in turn. The biotin and streptavidin form high-intensity non-covalent binding. After sufficient washing, the substrate TMB is added for colour development. TMB is converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The colour shading is proportional to the FGF6 content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of FGF6 in the sample was calculated by plotting the standard curve.

Product Composition

Reagents	Specifications（96T）	Storage Conditions
Antibody-Coated Strip	8×12	-20℃
Standard	2 tubes	-20℃
S1 Standard/Sample Dilution Buffer	45 ml×1 bottle	-20℃
Detection Solution A	120µl×1 tube	-20℃
Detection Solution B	120µl×1 tube	-20℃
Washing Buffer (Concentrated 30×)	20ml×1 bottle	2-8℃
TMB Substrate (Avoid direct light)	9ml×1 bottle	2-8℃
Stop Solution	6ml×1 bottle	2-8℃

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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℃, 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. 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 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 1000 x g. 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 1000 x g. Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.

3. **Other biological fluids**: When testing secreted components, collect using sterile tubes, centrifuge at 1000 x g for about 20 minutes, and collect the supernatant.

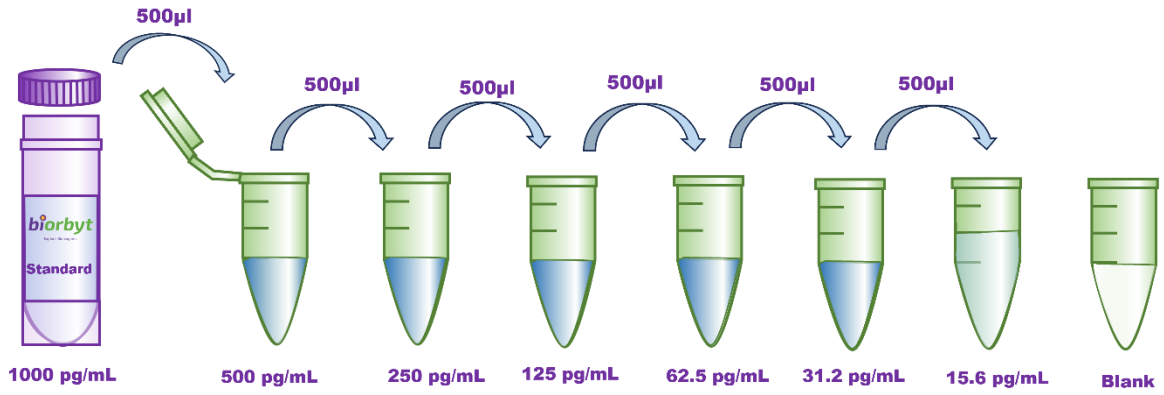
4. If the sample cannot be tested immediately, dispense it according to the minimum amount of use, and store it in -20℃ to -70℃ to avoid repeated freezing and thawing. Avoid haemolytic or hyperlipidaemia samples.

Reagent Preparation

1. Equilibration: Please warm the reagent kit and the sample to 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 (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 1000pg/ml, take 6 EP tubes, add 500ul Standard/Sample Dilution Buffer (S1) each EP tube, 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 (S1) 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, gently shake by hand a few times or briefly centrifuge to bring any liquid deposited on the tube walls or bottle cap down to the bottom of the tube. Just before use, dilute each solution 1:100 with the diluent (e.g., 10 µL of Detection Solution A in 990 µL of diluent) and mix thoroughly. Prepare the total amount needed for each experiment (100 µL per well) based on prior calculations, and prepare an extra 0.1–0.2 mL to ensure sufficient volume.

Operation Steps

1. Sample addition: According to the required amount for the experiment, take out the corresponding antibody-coated plates, and add **100 µl** per well of the prepared standards, standard zero point, and samples to be tested at the bottom of the wells.

2. Incubation: Seal the plate with sealing tape and incubate at 37°C for 120 minutes.

3. Discard the liquid, flick dry, no washing required.

4. Add Detection Solution A working solution (prepared freshly before use): add **100 µl** per well.

5. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 minutes.

6. Washing: Carefully remove the sealing tape, discard the liquid, flick dry, fill each well with **350 µl** washing solution, let stand for 1-2 minutes, then discard; repeat this process **3 times**, and finally blot dry on absorbent paper.

7. Add Detection Solution B working solution (prepared freshly before use): add **100 µl** per well.

8. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 minutes.

9. Washing: Follow the same washing procedure as described above (step 6), wash the plate 5 times.

10. Color development: Add **90 µl** of substrate solution to each well, seal the plate, and incubate at 37°C for 15-25 minutes.

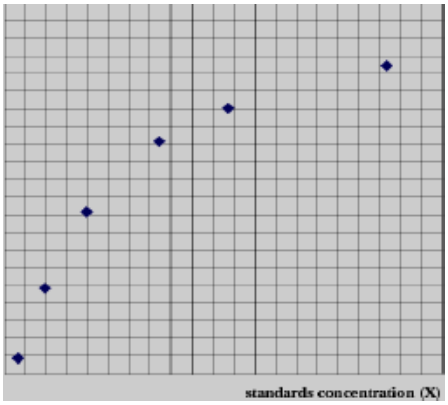
11. Termination: Add **50 µl** of stop solution to each well (the color changes from blue to yellow).

12. Measurement: Use a microplate reader to measure the absorbance (OD value) at 450 nm for each well. The measurement should be performed 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 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 FGF6 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

7.4 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
	Improper incubation temperature and time in the experiment	Determine the appropriate incubation temperature and time for each test step

High background	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

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