PROMPT
Proteins for Research

ISO 9001:2015 ISO 13485:2016

Zebrafish LZM (Lysozyme) ELISA Kit

Cat: OPK10068

For research use only. Not intended for diagnostic use.

Sensitivity: Minimum detected dose is less than 10ng/mL

Detection Range: 15 - 480ng/mL

Recovery Rate: The Recovery Rate is between 85% and 115%

Specificity: This kit recognizes natural and recombinant Zebrafish LZM (Lysozyme) without crossing with structural analogs

Stability: Unopened kits are kept at 2- 8°C and expired kits must not be used.

Kit Components & Storage

The standards have concentrations of 2.5, 5, 10, 20, 40, 80pg/mL respectively. Store the kit at 2-8°C, valid for 6 months.

Reagents	Quantity		Storage Condition After Opening
	48T	96T	
Pre-coated enzyme plate	48 wells	96 wells	2-8°C, 14 days
Standard	0.3mL × 6 tube	0.3mL × 6 tube	2-8°C, 14 days
Sample Dilution	3mL	6mL	2-8°C, 180 days
HRP-labeled antibodies	5mL	10mL	2-8°C, 14 days
Colour-producing substrate A	3mL	6mL	2-8°C, 180days
Colour-producing substrate B	3mL	6mL	2-8°C, 180days
Termination fluid	3mL	6mL	2-8°C, 180days
20× washing solution	15mL	25mL	2-8°C, 180days
Plate cover	2 pieces	2 pieces	2-8°C

Special Explanation

1. Before use, please check whether the label and quantity of the reagent in the kit are consistent with the table.
2. If the components of the kit need to be used again, please make sure that they are not contaminated after the last use.
3. The enzyme label plate has not been used for a single time. Remember to seal it and store it at 2-8°C.

Materials Required, Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2μL to 1mL volumes.

Safety Notes

1. This kit is only used for lab research and development and should not be used for humans or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with reagents. In case of contact, wash thoroughly with water.

Notes

1. It is for scientific research only and shall not be used for clinical diagnosis.
2. Used within the validity period marked by the kit, expired products shall not be used.
3. Do not mix with other manufacturers' kits or components, use the Sample Dilution that is equipped with the kit.
4. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately before re-measurement.
5. The heterophilic antibodies such as human anti-mouse in the sample to be tested will interfere with the detection results. Please excrete this factor before testing.
6. The test results obtained by other methods are not directly comparable to the test results of this kit.
7. Please wear experimental clothes and latex gloves to protect yourself during the test. Especially when testing blood or other body fluid samples, please follow the National Biological Laboratory Safety Protection Regulations.
8. Incubate strictly in accordance with the prescribed time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Refrigerate immediately after use.
9. Incorrect washing of the board can lead to inaccurate results. Make sure to suck the liquid in the pores as much as possible before adding the substrate. Do not let the micropores dry out during incubation.
10. Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
11. The substrate colour-producing liquid should be colourless or very light.
12. Avoid cross-contamination of reagents and specimens to avoid causing erroneous results.
13. Avoid direct exposure of strong light during storage and incubation.
14. The microplate reader used for detection needs to be installed with a filter that can detect wavelengths of 450 ± 10nm, with an optical density range between 0-3.5 . It is recommended to warm up 15 minutes in advance when using it.
15. The EP tube and suction head used in the test are both used once and are strictly prohibited from being mixed.

Test Principle

This kit uses double antibody Sandwich enzyme-linked immunosorbent assay (ELISA). To the microporous enzyme plate precoated with anti-Zebrafish LZM (Lysozyme) antibody (solid phase antibody), Zebrafish LZM (Lysozyme) calibrator and sample to be tested were added, and then HRP-labeled anti-Zebrafish LZM (Lysozyme) antibody (enzyme-labeled antibody) was added. After incubation and thorough washing, the unbound components were removed, and a sandwich complex of solid-phase antibody-antigen-enzyme-labeled antibody was formed on the solid phase surface of the microplate. Substrate A and B are added, and the substrate is catalyzed by HRP, and the substrate is produced, which is finally converted to yellow under the action of the Termination fluid (acid solution). The absorbance (OD value) was measured at a wavelength of 450nm on the microplate reader, and the absorbance (OD value) was positively correlated with the concentration of Zebrafish LZM (Lysozyme) in the sample to be tested. Fitting the calibrator curve, the concentration of Zebrafish LZM (Lysozyme) in the sample can be calculated.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C and then centrifuging at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 × g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900µL lysis buffer is added in 100mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Cell culture supernatant – Centrifuge to remove the precipitate, analyze immediately or aliquot it and store it frozen at -20°C

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. Before use, all components must be re-warmed for at least 60 minutes to ensure full re-warming to room temperature.
2. Concentrated washing solution: The concentrated washing solution taken out of the refrigerator will produce crystallization, which is a normal phenomenon. The crystallization is completely dissolved by heating in a water bath. The concentrated washing solution and distilled water are diluted at 1:20, that is, 1 part of the concentrated washing solution, and 19 parts of the distilled water are added.
3. Substrate: Colour-producing substrate A and B, mix thoroughly at 1:1 volume before use, and use within 15 minutes after mixing.

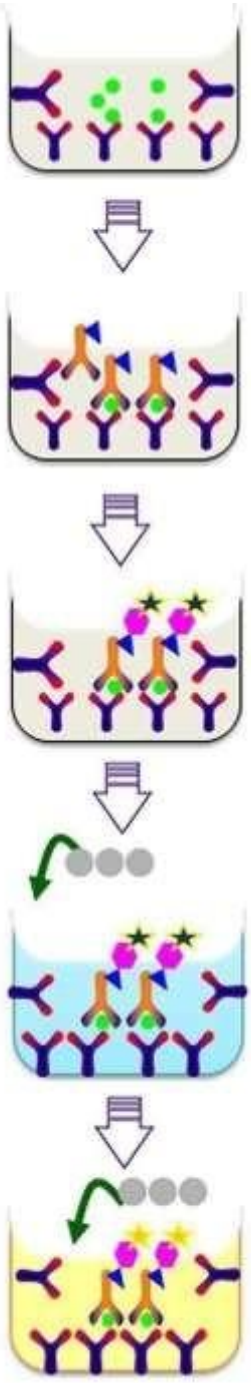
Assay Procedure

All reagents and components are first restored to room temperature and are recommended to make compound wells.

1. Prepare the working liquid of various components of the kit according to the method described in the previous instructions.
2. Remove the required slats from the aluminum foil bag and seal the remaining slats with a self-sealing bag and put them back into the refrigerator.
3. Set up standard product wells, 0-value wells, blank wells and sample wells. Add 50µL of standard products of different concentrations to each standard product wells, add 50µL of Sample Dilution for 0-value wells, and do not add blank wells, add 50µL of sample to be tested.
4. In addition to the blank wells, standard wells, 0 value wells and sample wells, add 100µL of horseradish peroxidase (HRP) labeled detection antibody.
5. Cover the reaction plate with a sealing film, incubate at 37°C water bath or constant temperature box away from light for 60 minutes.
6. Remove the sealing membrane, discard the liquid, pat dry on the absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, pat dry on the absorbent paper, and repeat this 5 times. If you use an automatic plate washing machine, please follow the plate washing machine operating procedure and add a program of soaking for 30 seconds to improve the detection accuracy. After washing the plate, pat the reaction plate thoroughly on clean paper that does not remove shaved content before adding substrate.

   (Tip: To obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please perform the next step immediately and do not let the microplate dry.)
7. Mix Colour-producing substrate A and B well in a 1:1 volume and add 100µL of substrate mixture to all wells. Cover the reaction plate with a sealing film, incubate at 37°C water bath or a constant temperature box for 15 minutes away from light.
8. Add 50µL of the Termination fluid to all wells and read the absorbance (OD value) of each well on a 450nm wavelength microplate reader.

Summary



1. After adding 50µL of standard working fluid or sample to the corresponding plate well, add 100µL HRP enzyme-labeled antibody working fluid to each well immediately, and incubate at 37°C for 60 minutes.

2. Discard the liquid in the plate add wash the plate 5 times

3. Add 100µL of colour producing substrate mixture to each well

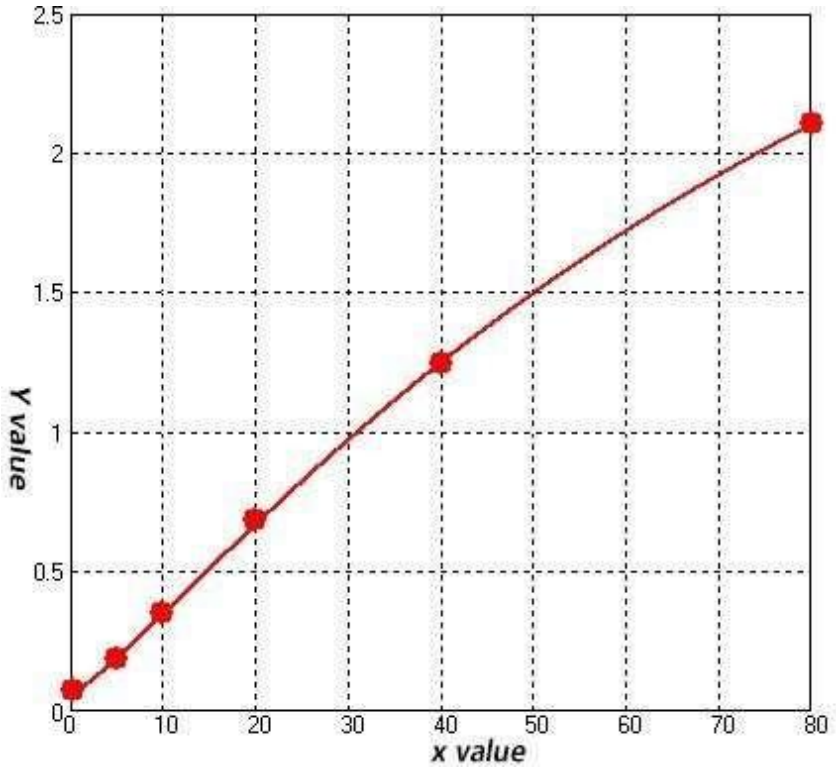
4. Add 50µL Termination fluid to each well

5. Immediately read at 450nm wavelength and process data

Calculation of Results

1. Use the concentration of the standard substance on the X-axis and the corresponding absorbance (OD value) on the Y- axis. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value, use the equation to calculate the concentration value of the sample. (Calculate using ELISA Calc software. It is recommended to use four-parameter fitting for the standard curve, but it is not the only fitting method)
2. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to determine the final value of the sample concentration.

   Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only.



Note: this graph is for reference only

Problem Analysis

If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you.

Fast Questions and Answers

Problem description	Possible reasons	Corresponding countermeasures
Standard curve gradient difference	Incorrect liquid aspiration or addition	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes and the amount of liquid added to each well
Very weak or colourless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspiration and addition process to ensure that all reagents are added in sufficient order and in sufficient quantities.
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid colour development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter settings on the microplate reader
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the detection antibody is too high	Use the recommended dilution factor
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked; whether the washing solution provided in the kit is used
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to instructions
	Not terminated before reading	Termination fluid should be added to each well before OD reading

Statement

1. Due to the current conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.
2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.
3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. The user should fully consider the possible usage of the sample and reserve sufficient samples before use.
4. In order to achieve good experimental results, please only use the reagents provided in our company kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may result. Please read the instructions carefully and adjust the instrument before the experiment.
6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.
7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.
8. This kit has not been compared with similar kits from other manufacturers or products that detect the same target substance using different methods, so inconsistent test results cannot be ruled out.
9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability