Pannarase Nuclease ELISA Kit

Detection principle:

This ELISA kit uses enzyme-linked immunosorbent assay technology based on the double antibody sandwich method. Coat the monoclonal antibody against Pannarase on the enzyme-linked plate, and add gradient diluted standards and pre diluted samples respectively. Pannarase in the standards and samples will fully bind to the coated antibodies on the enzyme-linked plate. After washing the plate, add biotinylated anti Pannarase antibodies, which will specifically bind to the standards captured by the coated antibodies on the plate and Pannarase in the samples; After washing the plate, streptavidin labeled with horseradish peroxidase (HRP) was added, and biotin and streptavidin exhibited strong non covalent binding; Then after washing the plate, add the chromogenic agent substrate TMB. If there are different concentrations of Pannarase in the sample in the reaction well, HRP will make the colorless TMB turn into blue substances of different depths (positively correlated). After adding the termination solution, the reaction well will turn yellow; Finally, the absorbance (OD) of the reaction well sample is measured at the point where λmax = 450 nm（OD = 450 nm） and the Pannarase in the sample is proportional to OD. The concentration of Pannarase in the sample can be calculated by drawing a standard curve using four parameter fitting software.

Note:

1. The kit should be used within its validity period, please do not use expired reagents.
2. The kit should be stored in a 2-8 ℃ refrigerator when not in use. Please discard the standards that have been redissolved but have not been used up.
3. Before using the kit, please restore it to room temperature for 30 minutes and fully mix the various components and samples prepared in the kit.
4. In the experiment, standards and samples should be subjected to double well testing, and the order of adding reagents should be consistent.
5. To avoid cross contamination, please use a disposable pipette tips,sealing film, and clean plastic container during the experiment.
6. The volume of concentrated biotinylated antibodies and concentrated enzyme conjugates is relatively small, and trace amounts of liquid may get onto the tube wall and bottle cap during transportation. Before use, please centrifuge (5-10 seconds) to concentrate the liquid on the tube wall at the bottom of the tube. When taking it, please use a pipette to carefully blow it several times.
7. Please do not use reagents from other source to replace a single component in this kit, except for concentrated washing solution and termination solution that can be used interchangeably.
8. To ensure accurate results, standard curves need to be made for each test.
9. If the detection concentration of the target substance in the sample is higher than the highest value of the standard substance, please dilute the sample appropriately before testing. It is recommended to conduct a pre experiment before the formal experiment to determine the dilution ratio.

Tips:

The termination solution in the kit is an acidic solution. Operators should wear gloves and pay attention to protection during use. During operation, it is also important to avoid contact with skin and eyes. If contact occurs accidentally, rinse with plenty of water; When testing blood samples and other body fluid samples, please follow the relevant safety protection management regulations of the national biological laboratory.

Kit composition and storage:

Composition	Size（96T）	Storage condiction
Antibody pre coated enzyme labeling plate	8*12	2-8℃
Standard product	2	-20 ℃
SR1 Standard/Sample Dilution	16 mL/bottle	2-8℃
Concentrated biotinylated antibody	60 μL(200×)	2-8℃
SR2 Biotinylated antibody diluent	16 mL/ bottle	2-8℃
Concentrated enzyme conjugates (protected from light)	120 μL(100×)	2-8℃
SR3 Enzyme conjugate diluent	16 mL/bottle	2-8℃
Washing Concentrate（20×）	30 mL/botlle	2-8℃
Coloring substrate (dark)	12 mL/botlle	2-8℃
Stop solution	12 mL/botlle	2-8℃
Sealing tape	4 pieces	
Instruction for use	1	

Self provided experimental equipment

1、Enzyme reader (main wavelength 450nm, reference wavelength 630nm)

2、High precision pipette and disposable tip：0.5- 10,　2-20，20-200，200-1000μL

3、Washing machine or bottle

4、Double distilled water, deionized water, measuring cylinder, etc

Reagent preparation:

1. Reagent rewarming: First, place the reagent kit and the sample to be tested at room temperature 30 minutes before the experiment. If crystals appear in the concentrated washing solution, please place it in a 37 ℃ warm bath until all crystals are dissolved.

2. Preparation of washing solution: Calculate the volume of diluted washing solution in advance, and then dilute 20 times the concentrated washing solution to 1 time the application solution with double distilled water or deionized water. Store the unused concentrated washing solution in a 4 ℃ refrigerator.

3. Standard gradient dilution: Add 2 ml of standard/sample diluent to the freeze-dried standard (Concentration of 10 ng/mL), let it stand for 10 to 30 minutes until it is completely dissolved, twofold diluted at the following concentrations:10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 ng/ml. 10 ng/ml is the highest point concentration of the standard curve, and the standard/sample dilution is used as the zero point (0 ng/ml) of the standard curve.The standard stock solution that has been re dissolved but has not been used up should be discarded.

4. Biotinylated antibody working solution: Calculate the required amount for the experiment in advance, and dilute 200 times the antibody concentration to 1 time the application working solutionwith the detection dilution solution (SR2) (thoroughly mix before dilution). Please add it to the reaction well within 30 minutes.

The dilution method for biotinylated antibody working solution is as follows:

Plate	Concentrated biotinylated antibody(1:200)：μL	Antibody HRP diluent：μL
2	**10**	**1990**
4	**20**	**3980**
6	**30**	**5970**
8	**40**	**7960**
10	**50**	**9950**
12	**60**	**11940**

5. Enzyme binding working solution: Prepare according to the required amount for each experim ent. Dilute 100 times the concentrated enzyme binding solution with enzyme binding diluent (SR3) to 1 time the application working solution (centrifuge before dilution). Please use within 30 minutes

The specific dilution method for enzyme conjugate working solution is as follows:

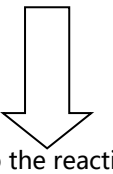
Plate	Concentrated enzyme conjugates(1:100):μL	Antibody HRP diluent:μL
2	**20**	**1980**
4	**40**	**3960**
6	**60**	**5940**
8	**80**	**7920**
10	**100**	**9900**
12	**120**	**11880**

6. Washing method:

- Automatic plate washing: shake off the liquid in the enzyme labeled plate hole, pat it dry on a thick layer of absorbent paper, and inject a washing solution of 300 μL/hole. The injection and extraction interval is 60 seconds,and the plate is washed 4 times
- Manual plate washing: shake off the liquid in the enzyme labeled plate hole, pat it dry on a thick layer of absorbent paper, and inject a washing solution of 300 μL/hole. After standing for 60 seconds, shake off the liquid in the enzyme label plate hole, pat dry on thick layers of absorbent paper, and wash the plate 4 times.

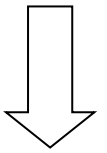
Test Step:

Take out the kit 30 mins before test and restore it to room temperature. Before adding the stan dard/sample, please wash the plate twice and shake it dry



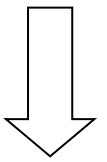
Add 100 μL standard and test samples to the reaction well, sealed the plate and incubate at room temperature (25 ± 2℃) for 120 minutes

Clap and wash the plate 4 times



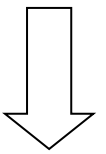
Add 100μL of biotinylated antibody working solution to the reaction well, seal the plate, and incubate at room temperature (25 ± 2℃) for 60 minutes

Clap and wash the plate 4 times

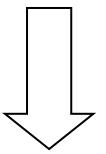


Add 100μL of enzyme conjugate working solution to the reaction well, seal the plate, and incubate at room temperature (25 ± 2℃) for 30 minutes

Clap and wash the plate 4 times



Add 100μL of chromogenic substrate to the reaction well.After sealing, incubate at room temperature (25 ± 2 ℃) for 10-15 minutes (depending on laboratory temperature)



Add 50μL of termination solution and measure the OD value with a microplate reader at a wavelength of 450nm within 5 minnutes

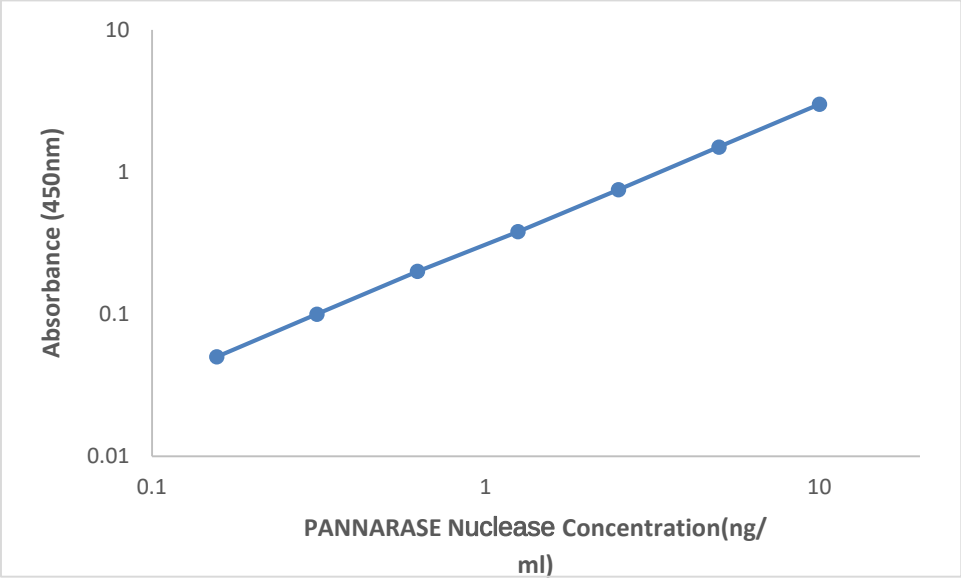
Result judgment:

1. Measure the OD value with a microplate reader at a wavelength of 450nm.Select dual wavelength detection with a reference wavelength of 630 nm. If dual wavelength detection is not available,please subtract the 630 nm OD measurement value from the 450 nm OD measurement value.
2. Calculate the average OD value of the standard and sample: The OD value of each standard and s ample should be subtracted from the OD value of the zero hole.
3. Using the standard concentration as the horizontal axis and the absorbance OD value as the vertic al axis, draw a standard curve using software. The content in the sample can be converted from the sta ndard curve to the corresponding concentration through the corresponding OD value.
4. If the OD value of the sample is higher than the upper limit of the standard curve, it should be dil uted appropriately and retested. Calculate the concentration by multiplying it by the dilution factor.

Parameters:

Data and standard curve

Standard concentration (ng/ml)	OD value 1	OD value 2	Average value	Correction
0	0.019	0.02	0.0195	-
0.15625	0.32	0.342	0.331	0.3115
0.3125	0.547	0.569	0.558	0.5385
0.625	0.868	0.895	0.8815	0.862
1.25	1.355	1.298	1.3265	1.307
2.5	1.817	1.835	1.826	1.8065
5	2.173	2.145	2.159	2.1395
10	2.56	2.614	2.587	2.5675



This figure is for reference only and the sample size should be calculated based on the standard curve drawn for the current test standard.

FAQ & Solutions

Questions	Possible causes	Solution
High background or high negative control value	Insufficient board washing	Inject detergent into the reaction hole for thorough washing, and thoroughly pat dry the liquid in the hole
	Excessive enzyme conjugates	Check the enzyme dilution. Dilute according to the dilution indicated in the instructions
	Substrate pollution	Before adding the substrate, check if it is transparent and colorless. Do not use a substrate that has turned blue
	Negative control well contaminated with positive control	Be careful not to spill the washing solution out of the hole during washing, so as not to connect the liquid in the negative and positive control holes together
	Mixing of different batches of reagents	Check the batch number of reagents, do not use different batches of reagents
Weak color signal	Reagent expired	Check the validity period of the reagent kit
	Incubation time is too short	Incubate according to the time specified in the instruction manual
	Reagent contamination	Check if the reagent is contaminated, do not use contaminated reagents
	Mismatch of ELISA reader filter	Check if the ELISA reader setup and filter match
	Insufficient balance of kit	Ensure that the reagent kit is balanced to room temperature before testing
	Insufficient color rendering time	Increase substrate color rendering time
No color signal	Missing detection of antibodies, enzymes, or chromogenic agents	Check the test operation process and repeat the test
	Enzymes contaminated with sodium azide	Please prepare new reagent
	Incorrect sequence of reagent addition	Check and review the addition sequence, process, and repeated tests of the test
The standard curve is good, but there is no signal from the sample hole	Low target content in the sample or no target in the sample	Set up positive control and repeat experiments
	Sample matrix effect impact detection	Retest after diluting the sample again
The standard curve is good, but the sample signal is on the high side	The content of the analyte in the sample exceeds the range of the standard curve	Retest after diluting the sample again
Edge effect	Uneven incubation temperature	During incubation, use new sealing tape every step to avoid incubating in areas with significant temperature changes, and do not stack reaction plates