Human Perforin ELISA kit

E22-HC154

Catalog No.:E22-HC154.96; E22-HC154.48

Size: 96 Tests;48 Tests;

EnoGene® human Perforin ELISA kit is suitable for quantitative determination of natural and recombinant human Perforin concentrations in human serum, plasma or cell culture supernatant in vitro. For scientific research only, please consult other special body fluid specimens. Please read the instructions carefully and check the composition of the reagent before use.

Kit composition:

The name According to	**E22-HC154.48 specifications**	**E22-HC154.96 specifications**	**Storage**
Antibody precoated enzyme labeling plate	8 x 6	8 x 12	4 ℃
Freeze-dried standard	1 vial- 2 ng/vial	2 vials- 2 ng/vial	4 ℃
Standard & specimen universal diluent	1 bottle 12 ml	1 bottle 20 ml	4 ℃
Biotinylated antibody (concentrated)	1 piece (see label for specifications	1 piece (see label for specifications	4 ℃
Biotinylated antibody dilution soution	1 bottle 10 ml	1 bottle 16 ml	4 ℃
Enzyme conjugates (concentrated)	1 piece (see label for specifications	1 piece (see label for specifications	4 ℃ (dark)
Enzyme conjugates dilution soution	1 bottle 10 ml	1 bottle 16 ml	4 ℃
Detergent solution (20×, Concentrated)	1 bottle 25 ml	1 bottle 50 ml	4 ℃
Color developing substrate (TMB)	1 bottle 6 ml	1 bottle 12 ml	4 ℃ (dark)
Reaction termination Buffer	1 bottle 6 ml	1 bottle 12 ml	4 ℃

Plate sealing tape	three	six	
Product manual	1	1	

Note: See the batch number of all reagents on the label.

Detection principle:

A double antibody sandwich method was used for the ELISA kit. Anti-human Perforin monoclonal antibody was coated on the plate. Human Perforin in the samples and the standard was binding to the monoclonal antibody and the free components were removed. Biotinylated antibody against human Perforin and horseradish peroxidase-labeled avidin were added, and biotin-avidin specific binding formed the immune complex. The free componens not binding to human Perforin antibody are washed away. Perforin, horseradish peroxidase causes the colorless agent to turn blue and the termination solution to turn yellow. When OD value was measured at 450 nm, the concentration of perforin was directly proportional to the OD 450 value. The concentration of perforin in in the sample could be calculated by drawing the standard curve.

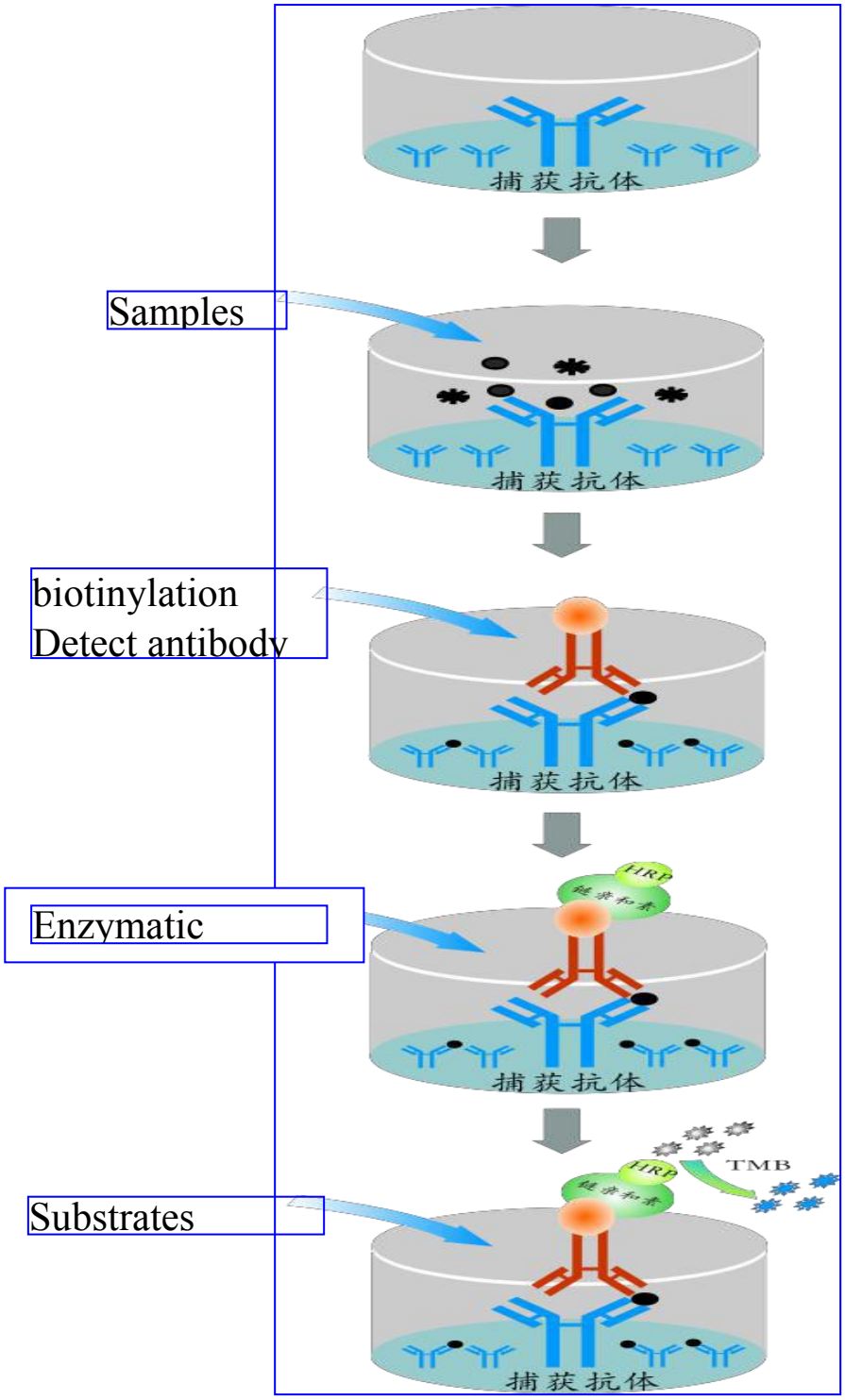
Self-preparedd test equipments (not provided, but can be purchased with assistance) :

1.ELISA machine (450 nm wavelength filter)

2.High precision liquid feeder and disposable suction head: 0.5-10 ul, 2-20 ul, 20-200 ul, 200-1000 ul.

3.37 ℃ thermostat, double steamed water or deionized water, coordinate paper, etc.

EnoGene® Double antibody Sandwich ELISA Schematic diagram:


捕获抗体
Samples
捕获抗体
biotinylation
Detect antibody
捕获抗体
HRP
Enzymatic
捕获抗体
HRP
TMB
Substrates
捕获抗体

Specimen Collection:

1.The test tube for collecting blood should be a disposable test tube free of pyrogen and endotoxin.
2.EDTA is recommended for plasma anticoagulants. Avoid using hemolysis and hyperlipidemia specimens.
3.The specimen should be clear and transparent, and the suspended matter should be removed by centrifugation.
4.If the specimens are not detected in time after collection, please pack them according to the amount used once and freeze them at -20 ℃.
In the refrigerator at -70 ℃, repeated freezing and thawing should be avoided and tested within 3-6 months.
5.If the concentration of the detected substance in your sample is higher than the maximum value of the standard substance, please make appropriate dilution according to the actual situation (pre-test is recommended to determine the dilution ratio).

Notes:

1.Please store the kit at 2-8 ℃ before use. Discard the redissolved standard if it is not used up.
2.Concentrated biotinylated antibodies, concentrated enzyme conjugates are small in size, bumpy in transport and may be inverted, causing the liquid to stick to tube walls or bottle caps. Therefore, before use, please shake the tube with your hand for a few times or 1000 rpm for 1 minute, so that the liquid attached to the tube wall or bottle cap can deposit to the bottom of the tube. Before taking, carefully blow the solution 4-5 times with a pipette to blend the solution.
3.The concentrated detergent solution taken from the refrigerator may crystallize, which is a normal phenomenon and is slightly heated to
Wash solution was prepared after crystallization was completely dissolved at 40℃. (The heating temperature should not exceed 50 ℃) The washing liquid should be at room temperature when used.
4.Components of kits of different batches cannot be mixed (except washing liquid and reaction stop liquid). Please consult Xinbosheng Biotechnology Co., LTD in advance.
5.Full and slight blending is especially important for the reaction result. It is better to use a microoscillator (using the lowest frequency). If there is no microoscillator, the enzyme label plate and frame can be lightly beaten by hand before the reaction.
6.Double hole testing is recommended for standard and sample testing during the test.
7.The test tube and suction head used in the test are disposable, it is strictly prohibited to mix between different liquids! **Otherwise, the test results will be affected.**
8.Please wear test suit and latex gloves for protection during the test. In particular, when testing blood or other body fluid samples, please follow the national Biological laboratory safety protection.
9.If it is used in several times, please prepare each component according to the amount needed, so as not to waste too much preparation and affect the follow-up test. Please seal the remaining plate holes with sealing tape, put them back in the foil bag, and use up within 2 months.

Preparation before test:

1.Please remove the kit from the refrigerator 20 minutes in advance to balance to room temperature.

2.Dilute 20× concentrated washing solution to 1× working solution with double steaming water. Put the unused ones back at 4 ℃.

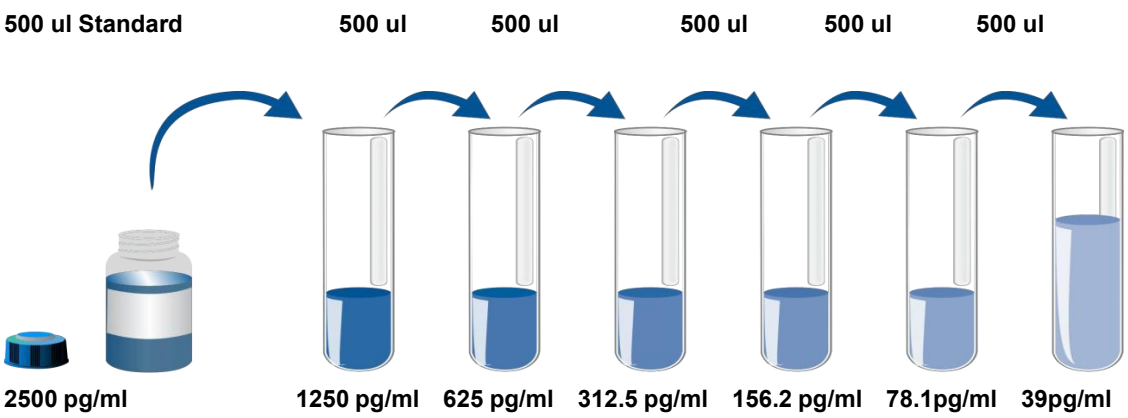
3.Standard substance: Add 0.8 mL of standard substance & specimen universal diluent to the freeze-dried standard substance, let stand for 15 minutes, after it is fully dissolved, mix gently (concentration: 2500 pg/ml). Then dilute as needed. (The following concentrations are suggested for the standard curve: 2500, 1250, 625, 312.5, 156.25, 78.1, 39, 0 pg/ml). For the Redissolved standard stock solution (2500 Pg /ml), if not used up, please discard.

4.Biotinylated antibody working solution: According to the amount required for the test, 20 minutes before use, use biotinylated antibody diluent to dilute 30× concentrated biotinylated antibody to 1× working solution. Day use. If the quantity of biotinylated antibody is insufficient due to too many experiments, biotinylated antibody can be purchased separately from Xinbosheng Company. (Antibody batch number required)

5.Enzyme binding working solution: According to the amount required for the test, 20 minutes before use, use enzyme binding diluent to dilute 30× concentrated enzyme binding solution to 1× working solution. Day use. If too many times of experiments result in insufficient concentration of enzyme binding, you can purchase the concentrated enzyme binding from Xinbosheng company separately. (Enzyme binding lot number must be provided)

Standard dilution method Legend:

Taking 500 ul/ tube as an example, the mother liquor of the redissolved standard was diluted by double ratio using standard & specimen universal diluent. The specific dilution method is shown in the figure below, and can also be diluted according to the actual dosage, such as 200 ul/ tube.



Washing method:

1.Automatic plate washer: the washing liquid required to be injected is 350 ul with an interval of 30-60 seconds between injection and suction. Wash the board 5 times.

2.Manual washing board: drain the liquid in the hole, pat dry on clean absorbent paper, add 350 uL washing liquid to each well, let stand for 30 seconds, then drain the liquid, pat dry on thick laminated absorbent paper. Wash the plate 5 times.

Operation steps:

1.Remove the test strip from the sealed bag that has been balanced to room temperature. The unused strip and desiccant should be put back into the foil bag to compress the self-sealing strip, seal the bag and put back to 4 ℃.

2.Add standard diluent to the blank hole, and add specimen or standard of different concentration (100 ul/ well) to the other corresponding holes. Seal the reaction hole with adhesive paper and incubate at 37 ℃ 90 minutes.

3.Prepare biotinylated antibody working fluid 20 minutes in advance.

4.Wash the board 5 times.

5.The blank hole was added with biotinylated antibody diluent, and the remaining hole was added with biotinylated antibody working liquid (100 ul/ well). The reaction hole was sealed with the new sealing board adhesive paper and incubated at 37 ℃ for 60 minutes.

6.Prepare the enzyme binding working fluid 20 minutes in advance. Place at room temperature (22-25 ℃) away from light.

7.Wash the board 5 times.

8.The blank well was added with enzyme binding diluent, and the remaining Wells were added with enzyme binding working liquid (100 ul/ m2)

Hole). The reaction hole was sealed with the new sealing board adhesive paper, incubated at 37 ℃, and incubated in darkness for 30 minutes.

9.Turn on the electrical source of the enzyme beacon, preheat the instrument, and set up the test procedure.

10.Wash the board 5 times.

11.Add color substrate (TMB)100 ul/ well, incubate at 37 ℃ in dark for 15 min.

12.Add reaction termination liquid 100 ul/ well and measure OD immediately after mixing 450 Value (within 3 minutes). Save the readings in the instrument and print a paper copy of the results.

13.After the end of the experiment, put the unused reagents back to the refrigerator at the specified storage temperature until the expiry date.

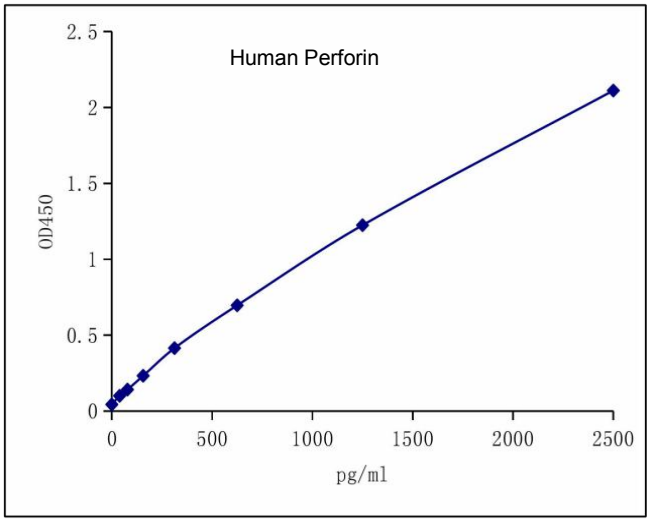
It is suggested to preserve the enzyme label plate and frame for future use.

Result Analysis:

1.The OD value of each standard and specimen shall be subtracted from the OD value of the blank hole.

2.The standard concentration is used as the abscissa and OD value as the ordinate, and the standard curve is drawn manually or by software. By OD value of the specimen, its concentration can be detected on the standard curve.

3.If the OD value of the specimen is higher than the upper limit of the standard curve, it should be recalculated after appropriate dilution, and the dilution ratio should be multiplied when calculating the concentration.



Note: This chart is for reference only. The content of Perforin in the sample should be calculated using the standard curve shown in the same test standard.

Sensitivity, specificity and repeatability:

●Sensitivity: Minimum measurable number of Perforin reached 19.5 pg/ml.

●Specificity: Not reaction with recombinant human IL-1,IL-2,IL-3,IFN-,TNF,Fas, FAS-L, TRAIL.

●Repeatability: In plate, coefficient of variation between plates <10%.

Human Perforin Background:

Perforin, also known as PRF or PRF1. Human Perforin signal peptide consists of 20 amino acids. After endoplasmic reticulum processing, mature Perforin is released, and its cytolytic activity is mainly located in the first 34 amino acids at the N-terminal. Structure and function of Perforin and its complement component 9 (C9) Similar, can form transmembrane tubules, non-specific lysis of a variety of target cells. Perforin is the main lysosomal protein in the lysosome and is the main effector molecule of the cytolysis mediated by T cells and natural killer cells. Perforin is primarily found in cytotoxic T cells (CTLs) and NK cells, these cells are degranulated and Perforin is inserted into the cell membrane of the target cell to form pores. Perforin is inserted into the cell membrane lysosome in an area called MACPF, which is homologous with the cholesterol dependent melting cytins derived from gram-positive bacteria.

References:

Yuqi Yang, Shuli Li, Guannan Zhu, et al. (2017). " A similar local immune and oxidative stress phenotype in vitiligo and halo nevus." Journal of Dermatological Science.

Analysis of EnoGene® ELISA COMMON Problems:

Question 1: High background or negative control value is high

Possible reasons	Advice
The negative control hole was contaminated with the positive control or sample	Do not spill the lotion out of the hole when washing.
Inadequate washing	Make sure that each hole is filled with liquid when washed, and make sure that the residual liquid is removed from the hole.
Excessive concentration of enzyme binding	Please check whether the enzyme conjugate is diluted as specified in the instructions
The incubation temperature is too high	Check incubator temperature is correct and stable
Exposure of substrates before use	It should be kept in the dark, away from light.
Stay too long in front of the board	Reading within 3 minutes after addition of termination fluid

Problem 2: The positive control value is low or low absorbance

Possible reasons for	Advice
The reagent is not balanced to room temperature	Before starting the experiment, the kit was balanced to room temperature
There is moisture in the bag	Mark the date of the first opening of the bag, seal the unused hole strips, and put in the desiccant.
There are inhibitors in the sample	Sodium azide inhibits HRP activity
The reagent was not well mixed before use	Mix the reagent before use
The washing steps are too drastic	Reduce the pressure on the washing system
After the experiment began, the micropores became dry	Do not interrupt the experiment. All the experiments should be completed continuously Steps.
The detection volume is small	Make sure the pipette has been corrected

Question 3: Edge effect

Possible reasons for	Advice
The evaporation	The reaction plate shall be sealed with plate sealant between each step.
Temperature inhomogeneity	Calibrate incubators and do not stack reaction plates.

Problem number four: The standard curve is not good

Possible reasons for	Advice
Diluting the standard at multiple ratio without blending	Each step of diluting the standard should be mixed well
Early to dilute	The standard is diluted at the point of use
The volume added is incorrect	A calibrated pipette is used to quickly and equally distribute the standard to the micropores.

Question 5: The standard curve is good, but the sample has no detectable signal

Possible reasons for	Advice
There is no detectable substance or very low detectable content in the samples	Set internal parameters and retry the experiment
Other substance influence/cover-up detection in the sample	Appropriate dilution to reduce interference of other substances, or series dilution to detect recovery.
The concentration of the detected substance in the sample is too high. The Hook effect causes false low values	The sample is diluted in appropriate multiples, and the concentration of the detected substance should be within the detection range of the kit.

Problem 6: Poor repeatability

Possible reasons for	Advice
There are bubbles in the micropores	Pop the bubbles with the tip of a needle
The reagent is not well mixed	Make sure to mix the reagent well
There is impurity or sediment in the sample	Pre-Centrifugation
The microporous coating surface was scratched by the suction head	Handle with care when adding liquid
Use used sealing tape	Each time the reaction hole shall be sealed with a new sealing plate adhesive