Instructions for Use of Human Granzyme B ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- GB001

Introduction

This kit uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method. Add human Granzyme B standard and test samples to the microtiter plate pre-coated with anti-human Granzyme B antibody, then add diluted biotin-labeled human Granzyme B detection antibody, finally add streptavidin-HRP to form the antibody + antigen + antibody-Biotin + SA-HRP complex, wash the plate and add TMB chromogenic solution for color development. TMB is converted from colorless to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The shade of yellow is positively correlated with the amount of human Granzyme B detected in the samples.

Assay range : 6.86-5000 pg/mL
Sensitivity : 1.12 pg/mL

Specification

96 T

Usage

It is applicable to the optimization of purification process of biological products, impurity control of intermediate process and release testing of final products.

Kit components

Components	Specification	Preparation
Standard	S0-S7, S	Prepare according to the instructions
Coated Plate	8 wells × 12 strips	Ready-to-use
Assay Buffer	12 mL x 1 bottle	Ready-to-use
Wash Buffer (10x)	50 mL × bottle	Make a 10-fold dilution with ultrapure water.
Detection Antibody	6 mL × 1 bottle	Ready-to-use
Streptavidin-HRP	12 mL × 1 bottle	Ready-to-use
TMB Substrate	12 mL × 1 bottle	Ready-to-use
Stop Solution	12 mL × 1 bottle	Ready-to-use
Sealing Film	5 pieces	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Storage and shelf life

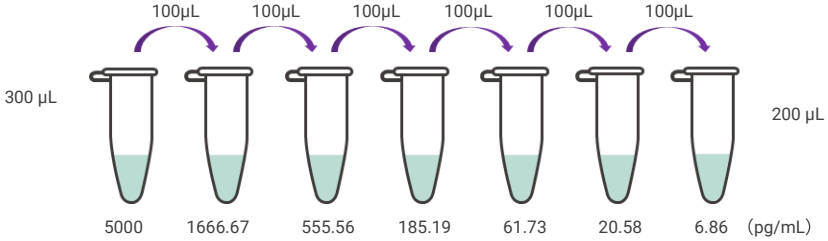
Sealed kits are valid for 12 months at 2-8℃.

Apparatus and materials to be prepared by the user:

- Plate reader
- Constant temperature oscillation incubator
- Pipette and tips
- Deionized water
- Absorbent paper
- Vortex shaker

Pre-experiment preparation

1. All reagents and samples to be tested should be restored to room temperature. All reagents are newly prepared and ready-to-use.
2. Preparation of 1× washing solution: Equilibrate the concentrated washing solution to room temperature, without crystallization. After mixing well, according to the usage volume, dilute 10x washing solution by 10 times with an appropriate amount of Deionized water at the ratio of 1:9, to obtain 1× washing solution.
3. Preparation of standards:
   - For serum and plasma samples, use ready-to-use standards S0 to S7.
   - For other types of samples, use the high-concentration standard S (50,000 pg/mL) for gradient dilution. Prepare 8 × 1.5 mL centrifuge tubes, labeled in order of standard concentration. Take 30 μL of standard S plus 270 μL of SPB (Sample Matrix, such as culture medium, diluent, self-made buffer, etc.), resulting in a solution concentration of 5,000 pg/mL. Add 200 μL of SPB to each centrifuge tube, and dilute the high-concentration standard of 5,000 pg/mL by a factor of 1:2 for gradient dilution. The sample diluent is used as the zero standard (0 pg/mL).



Operation procedures

All reagent components and samples to be tested should be restored to room temperature before use. Duplicate well assay is recommended for all standards and samples to be tested.
1. Preparation of reagents: Prepare all reagents to be tested, diluted standards and samples to be tested in advance.
2. Microplate strip determination: Calculate the microtiter strips required for the samples to be tested and standards, remove the microtiter strips from the aluminum foil bag, place the remaining microtiter strips back into the aluminum foil bag and seal the mouth of the bag, and store it at low temperature.
3. Add 50 μL detection buffer to each well.
4. Sample and test antibody incubation: Add the 50 μL of standards and the sample to be tested to each well, and ensure that the spot sampling is completed within 15 min. Add 50 μL of 1 x detection antibody to each well. Seal the plate with the sealing film and incubate in a 25℃ thermostatic incubator at 500 rpm for 1 hour.
5. Plate washing: Discard the liquid in the wells, add 1 × washing solution (300 μL/well) to wash the plate for 4 times, and pat dry the residual liquid in the microtiter plate.
6. Enzyme conjugate incubation: Add enzyme conjugate into microtiter plate with 100 μL/well, seal the plate with sealing film, place it into a thermostatic incubator, incubate at 500 rpm for 30 minutes at 25℃.
7. Plate washing: Same as Step 5.

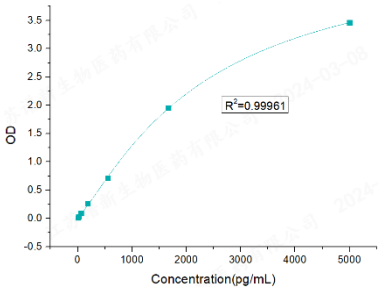
8. Add 100 µL of chromogenic substrate TMB to each well and incubate at room temperature for 15-20 minutes.
9. Termination: Add 100 µL stop solution into each well, and gently shake the microtiter plate until the color development is uniform.
10. Readings: Read the absorbance value at 450 nm/630 nm within 20 minutes. Take 450 nm as detection wavelength and 630 nm as reference wavelength.

Results processing

1. OD processing of the standard curve (See the following example, which is only for example purpose. Please refer to the actual measurement for details):

Standard concentration (pg/mL)	OD value (1)	OD value (2)	Mean value
5000	3.4680	3.4540	3.4610
1666.67	1.6813	1.4737	1.5775
555.56	0.7201	0.7241	0.7221
185.19	0.2709	0.2741	0.2725
61.73	0.0973	0.0932	0.0953
20.58	0.0396	0.0387	0.0392
6.86	0.0187	0.0273	0.0230
0	0.0085	0.0082	0.0084

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
3. Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. Please use the kit within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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