Version 02-11/24

User's Manual

Human Granzyme B ELISA Kit

REF **DEIA-PX0005**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

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

Principles of Testing

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 biotinlabeled 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.

Reagents And Materials Provided

1. Standard, lyophilized powder × 2 pieces, Gradient dilution with detection buffer
2. Coated Plate, 8 wells × 12 strips, Ready-to-use
3. Sample Diluent, 15 mL × 1 vial, Ready-to-use
4. Assay Buffer, 12 mL × 1 vial, Ready-to-use
5. Wash Buffer (10×), 50 mL × 1vial, Make a 10-fold dilution with ultrapure water.
6. Detection Antibody, 6 mL × 1 vial, Ready-to-use
7. Streptavidin-HRP, 12 mL × 1 vial, Ready-to-use
8. TMB Substrate, 15 mL × 1 vial, Ready-to-use
9. Stop Solution, 10 mL × 1 vial, Ready-to-use
10. Sealing Film, 5 pieces, Ready-to-use
11. Instructions for Use, 1 copy, Ready-to-use

Materials Required But Not Supplied

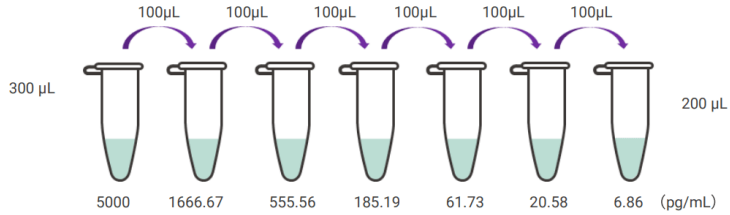
1. Plate reader
2. Thermostat plate shaker
3. Micro pipette and tips
4. Deionized water
5. Unused filter paper
6. Vortex shaker

Storage

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

Reagent Preparation

1. All reagents and samples to be tested should be restored to room temperature. All reagents are newly prepared and readyto 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 ultrapure water at the ratio of 1:9, to obtain 1x washing solution.
3. Preparation of standards: Prepare eight 1.5 mL centrifuge tubes and label them in turn according to the concentrations of the standards. Dissolve a vial of lyophilized standard with Diluent 1 according to the labeled amount, thoroughly dissolve and allow to stand for 10 minutes to obtain a solution concentration of 5000 pg/mL. Add 200 µL Diluent to each centrifuge tube, and the 5000pg/mL high concentration standard was diluted in a gradient of 1:2. Sample diluent used as zero standard (0pg/mL).



Assay Procedure

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°C 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°C.
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.

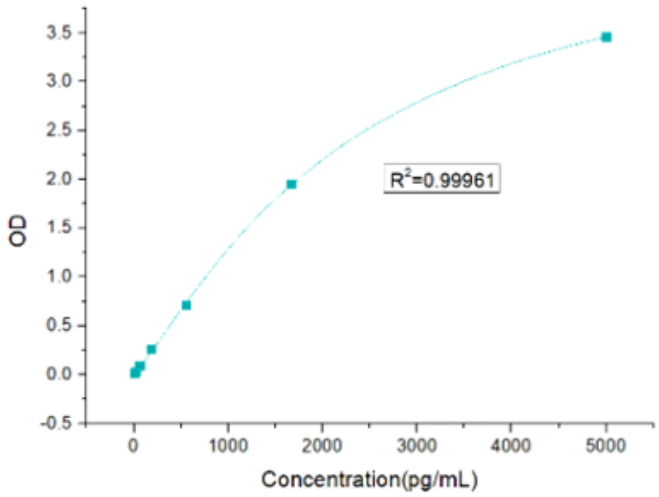
Calculation

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	3.4680	3.468
1666.67	1.9590	1.9590	1.959
555.56	0.7201	0.7201	0.7201
185.19	0.2709	0.2709	0.2709
61.73	0.0973	0.0973	0.0973
20.58	0.0396	0.0396	0.0396
6.86	0.0187	0.0187	0.0187
0	0.0085	0.0085	0.0085

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values.

Typical Standard Curve



Detection Range

6.86-5000 pg/mL

Sensitivity

1.12 pg/mL

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°C 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. The kit is intended for single use. Please use within the shelf life.