ClinMax™ Human Granzyme B ELISA Kit

Catalog Number: CEA-B033

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The kit is developed for quantitative detection of Granzyme B in human serum and cell culture supernates. It is intended for research use only (RUO).

BACKGROUND

Granzyme B is binds to the hepatocyte growth factor receptor to regulate cell growth, cell motility and morphogenesis in numerous cell and tissue types.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human Granzyme B by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Granzyme B Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Granzyme B Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of Granzyme B bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.

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4. Do not mix or substitute reagents with those from other kits or other lot number kits.

5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.

6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CEA033-C01	Pre-coated Anti- Granzyme B Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
CEA033-C02	Human Granzyme B Standard	20 µL	Liquid	2-8℃	2-8℃
CEA033-C03	Biotin-Anti-Granzyme B Antibody Con. Solution	100 µL	Liquid	2-8℃	2-8℃
CEA033-C04	Biotin-Antibody Dilution Buffer	8 mL	Liquid	2-8℃	2-8℃
CEA033-C05	Streptavidin-HRP Con. Solution	500 µL	Liquid	2-8℃, avoid light	2-8℃, avoid light
CEA033-C06	Streptavidin-HRP Dilution Buffer	15 mL	Liquid	2-8℃	2-8℃
CEA033-C07	20× Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
CEA033-C08	Sample Dilution Buffer	15 mL×2	Liquid	2-8℃	2-8℃
CEA033-C09	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
CEA033-C10	Stop Solution	6 mL	Liquid	2-8℃	2-8℃

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KIT STORAGE AND EXPIRATION DATE

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: 10 µL, 300 µL, 1000 µL;
37ºC Incubator;
Single or dual wavelength microplate reader with 450nm and 630nm filter;
Tubes: 1.5 mL,10 mL;
Timer;
Reagent bottle;
Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer

Dilute 50 mL 20×Washing Buffer with deionized or ultrapure water to 1000 mL.

1.2 Preparation of Biotin-Anti-Granzyme B Antibody Solution

Prepare Biotin-Anti-Granzyme B Antibody Solution by diluting 60 µL of Biotin-Anti-

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Granzyme B Antibody Con. Solution into 6 mL Biotin-Antibody Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

1.3 Preparation of Granzyme B Streptavidin-HRP Solution

Prepare Granzyme B Streptavidin-HRP Solution by diluting 240 µL of Granzyme B Streptavidin-HRP Con. Solution into 12 mL Streptavidin-HRP Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

2. Preparation of Standard curve

The concentration of reconstituted human Granzyme B Standard （CEA033-C02） is 250 µg/mL. Prepare Cm1 by adding 5 µL of the reconstituted human Granzyme B Standard to 1995 µL of Sample Dilution Buffer, mix gently well. Prepare Cm2 by adding 160 µL of the Cm1 to 840 µL of Sample Dilution Buffer, mix gently well. Label 8 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8. According to the following dilution scheme: Add 5 µL of Granzyme B Cm2 and 995 µL of Sample Dilution Buffer to tube Std.-1, shake gently to mix (Std.-1 =500 pg/mL). Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 500 µL of Sample Dilution Buffer into each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8). Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 250 pg/mL) Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-8. Sample Dilution Buffer serves as blank.

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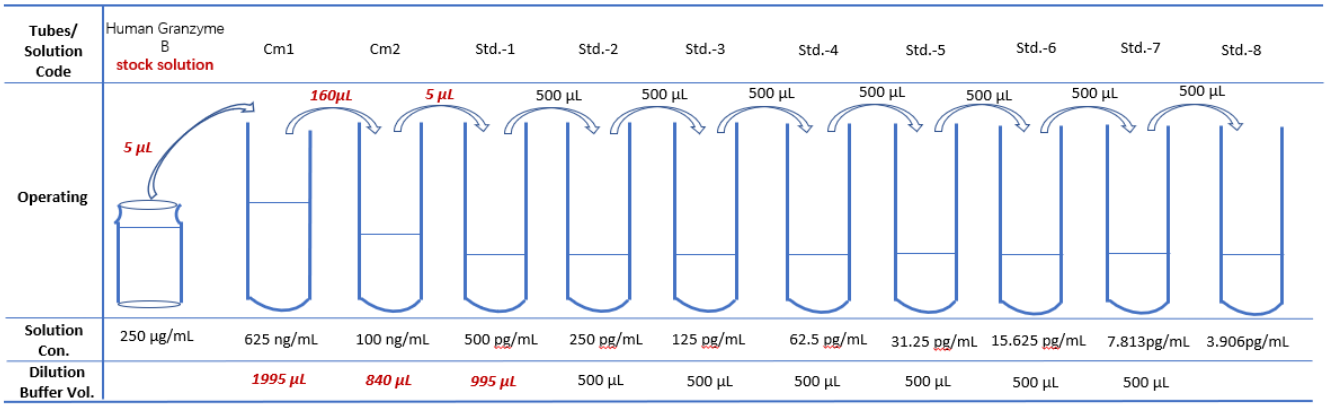

3. Add Samples

Add 50 µL Granzyme B Standard to each well, or add 50 µL samples to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **1.0 hour**.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for five times.

5. Add Biotin-Antibody Solution

Add 50 µL Biotin-Anti- Granzyme B Antibody Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **1.0 hour**.

6. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1×Washing Buffer: by aspirating or

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decanting, invert the plate and blot it against paper towels. Repeat the wash step above for five times.

7. Add Granzyme B Streptavidin-HRP Solution

For all wells, add 100 µL Granzyme B Streptavidin-HRP Solution. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **30 minutes, avoid light**.

8. Washing

Repeat step 4.

9. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.

10. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *the color in the wells should change from blue to yellow.*

11. Data Recording

Read the absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

Note*: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

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CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.

4. Detection range: 3.906 pg/mL-500 pg/mL.

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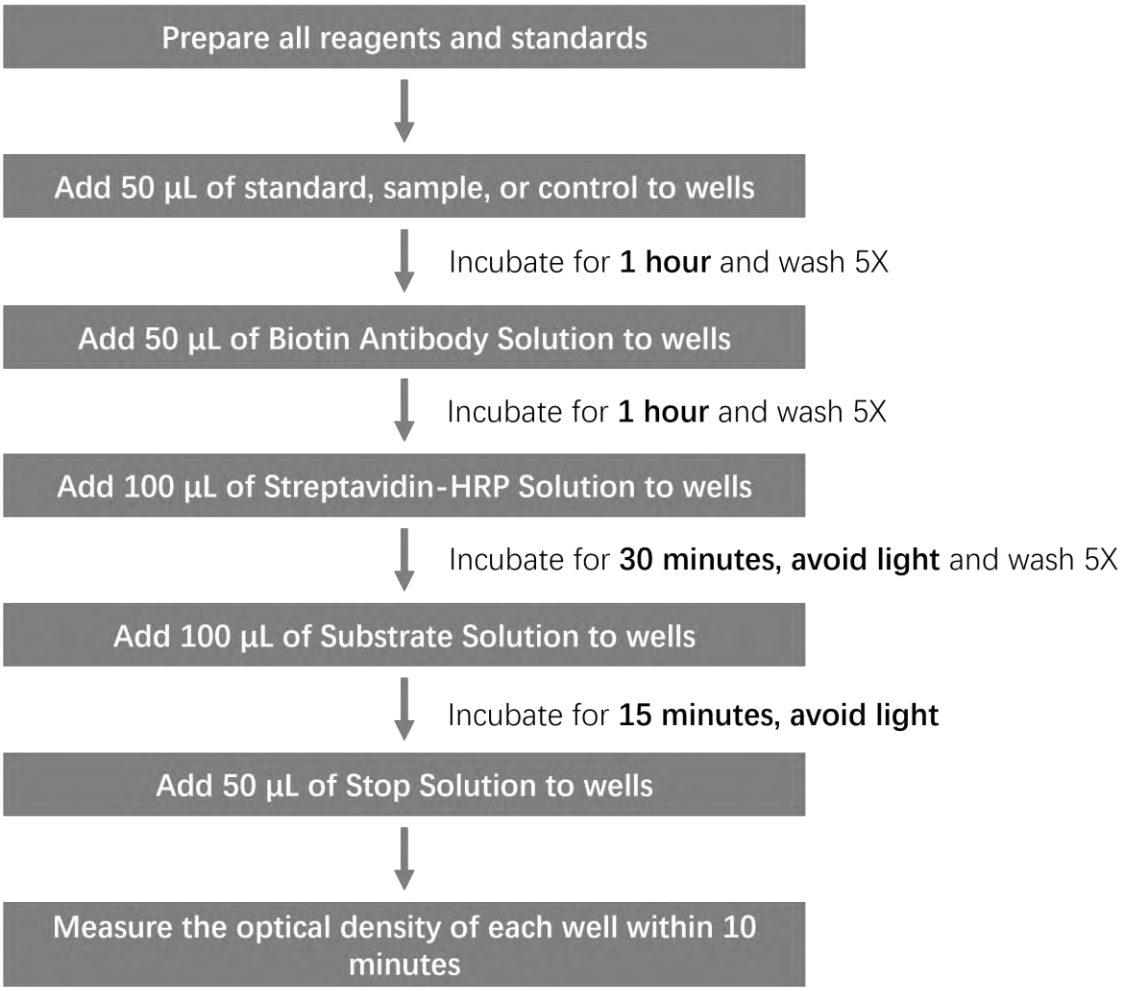
QUICK GUILD

Analyte: Granzyme B



NOTE: Incubation temperature is 18 ℃-25 ℃

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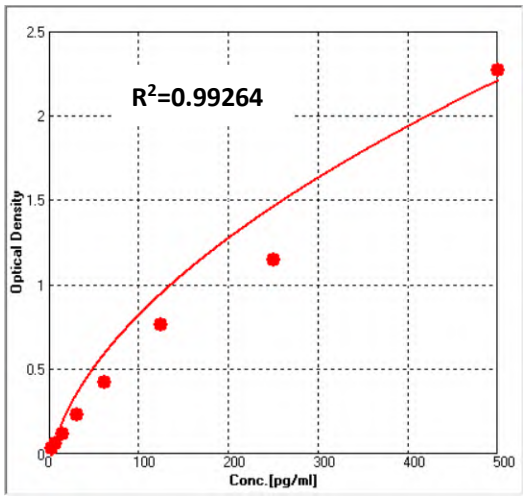
TYPICAL DATA

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Granzyme B Standard (pg/mL)	$OD_{450nm-630nm}$
500	2.267
250	1.145
125	0.764
62.5	0.423
31.25	0.231
15.625	0.120
7.813	0.060
3.906	0.030
Blank	0.010



SENSITIVITY

The minimum detectable concentration of human Granzyme B is 1 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

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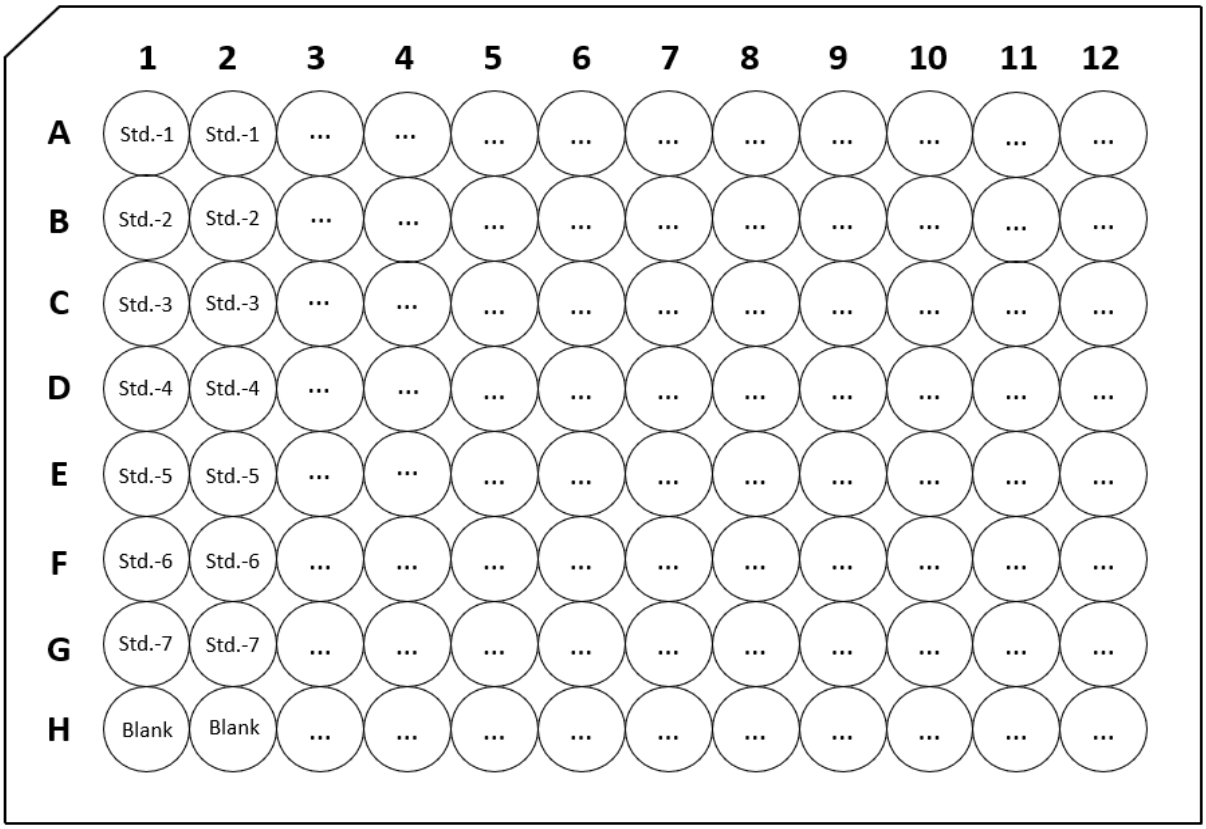
PLATE LAYOUT



Note: Sample Dilution Buffer serves as blank.

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay. * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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