

Human Granzyme B Sandwich ELISA Kit Datasheet

For the quantitative detection of Human Granzyme B concentrations in serum, plasma and cell culture supernatants.

General Information

| | |
|---------------------------|-------------------------------------|
| Catalogue Number | KE00121 |
| Product Name | Human Granzyme B Sandwich ELISA Kit |
| Species cross-reactivity | Human |
| Range (calibration Range) | 15.6-1000 pg/mL |
| Tested applications | Quantification ELISA |

Database Links

| | |
|-------------|--------|
| Entrez Gene | 3002 |
| SwissProt | P10144 |

Kit Components & Storage

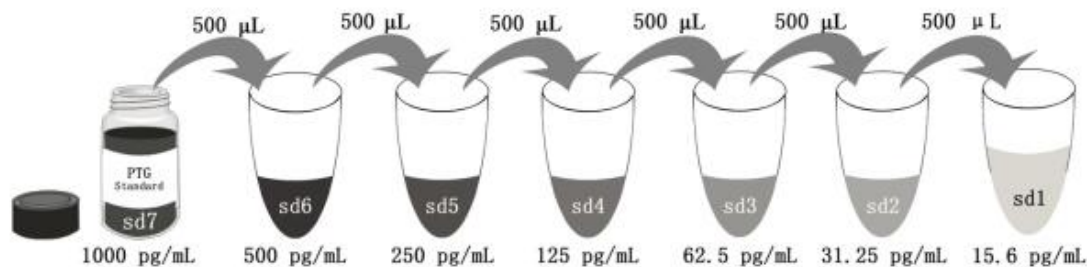
| | | |
|--|-----------|---|
| Microplate - antibody coated 96-well microplate (8 well × 12 strips) | 1 plate | Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay. |
| Protein standard - 2000 pg/bottle; lyophilized* | 2 bottles | |
| Detection antibody, biotinylated (100X) (100X) - 120 µL/vial | 1 vial | |
| Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial | 1 vial | |
| Sample Diluent PT 1-af - 30 mL/bottle | 1 bottle | |
| Detection Diluent - 30 mL/bottle | 1 bottle | |
| Wash Buffer Concentrate (20X) - 30 mL/bottle | 1 bottle | |
| Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle | 1 bottle | |
| Stop Solution - 12 mL/bottle | 1 bottle | |
| Plate Cover Seals | 3 pieces | |

NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-af is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 1-af in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



| | | | | | | | |
|---|---------|--------|--------|--------|--------|--------|--------|
| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| # µL of Sample Diluent PT 1-af | 2000 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

Product Description

KE00121 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Granzyme B ELISA kit is to be used to detect and quantify protein levels of endogenous Granzyme B. The assay recognizes human Granzyme B. An antibody specific for Granzyme B has been pre-coated onto the microwells. The Granzyme B protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for Granzyme B is added to detect the captured Granzyme B protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Granzyme B (GZMB) is also named as CGL1, CSPB, CTLA1, GRB and belongs to the Granzyme subfamily. This enzyme is necessary for target cell lysis in cell-mediated immune responses. The cytotoxic lymphocyte protease Granzyme B (GZMB) can promote apoptosis through direct processing and activation of members of the caspase family. Granzyme B also functions as processing protease of cytokines, including interleukin (IL)-18 and IL-1a, and extracellular matrix proteins, including fibronectin and various proteoglycans. Plasma Granzyme B concentrations were significantly higher in patients with Atopic dermatitis (AD) and psoriasis than in healthy controls, which indicated that Granzyme B may play an important role in the induction of intractable itch and dermatitis in AD.

Sample Preparation

The serum, plasma, cell culture supernatants may require proper dilution to fall within the range of the assay.

1:2 or 1:4 dilution is recommended for serum or plasma, 1:80 or 1:160 dilution is recommended for cell culture supernatants.

Safety Notes

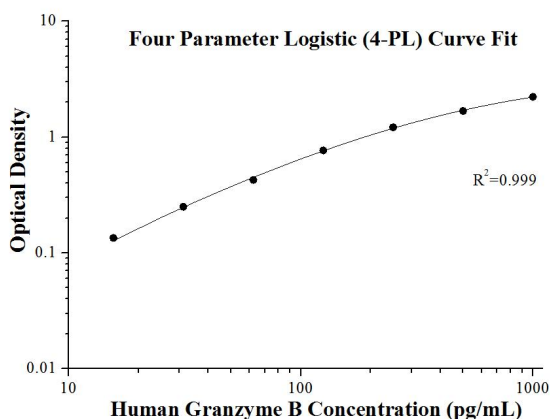
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

| Step | Reagent | Volume | Incubation | Wash | Notes |
|------|--|--------|------------|-------------|------------------------------|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



| (pg/mL) | O.D | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.037 0.035 | 0.036 | - |
| 15.6 | 0.174 0.167 | 0.171 | 0.135 |
| 31.25 | 0.294 0.278 | 0.286 | 0.25 |
| 62.5 | 0.474 0.449 | 0.462 | 0.426 |
| 125 | 0.798 0.806 | 0.804 | 0.766 |
| 250 | 1.234 1.266 | 1.25 | 1.214 |
| 500 | 1.679 1.75 | 1.715 | 1.679 |
| 1000 | 2.245 2.269 | 2.257 | 2.220 |

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | | Inter-assay Precision | | | | |
|-----------------------|----|--------------|------|-----|-----------------------|----|--------------|------|-----|
| Sample | n | Mean (pg/mL) | SD | CV% | Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 26.6 | 1.0 | 3.8 | 1 | 24 | 27.4 | 2.7 | 9.7 |
| 2 | 20 | 112.0 | 5.2 | 4.6 | 2 | 24 | 119.4 | 7.2 | 6.0 |
| 3 | 20 | 465.7 | 31.7 | 6.8 | 3 | 24 | 499.8 | 27.7 | 5.5 |

Recovery

The recovery of Granzyme B spiked to three different levels in four samples throughout the range of the assay in cell culture supernatants was evaluated.

| Sample Type | | Average % of Expected | Range (%) |
|---------------------------|---------|-----------------------|-----------|
| Human plasma | 1:2 | 86 | 78-90 |
| | 1:4 | 84 | 77-95 |
| Cell culture supernatants | 1:1,200 | 94 | 89-99 |
| | 1:2,400 | 87 | 78-98 |

Sample Values

Human serum/plasma -16 serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human Granzyme B in this assay. No medical histories were available for the donors used in this study. All samples measured between 11-19 pg/mL with a mean of 15 pg/mL.

Cell culture supernatants-Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 10 ug/mL PHA. Aliquots of the cell culture supernatants were removed on days 1 and 3 and assayed for levels of human Granzyme B.

| Condition | Day 1 (pg/mL) | Day 3 (pg/mL) |
|--------------|---------------|---------------|
| Unstimulated | 86 | 41 |
| Stimulated | 6,896 | 58,107 |

Sensitivity

The minimum detectable dose of Human Granzyme B is 2.6 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The Cell culture supernatants was initially diluted 1:40)

| | | Human plasma | Cell culture supernatants |
|------|----------------------|--------------|---------------------------|
| 1:2 | Average% of Expected | 77 | 97 |
| | Range (%) | 76-79 | 91-100 |
| 1:4 | Average% of Expected | 108 | 98 |
| | Range (%) | 100-124 | 94-100 |
| 1:8 | Average% of Expected | 98 | 94 |
| | Range (%) | 81-112 | 88-103 |
| 1:16 | Average% of Expected | - | 104 |
| | Range (%) | - | 96-113 |

References

1. Cullen S P, Adrain C, Lüthi A U, et al. Human and murine granzyme B exhibit divergent substrate preferences.[J]. Journal of Cell Biology, 2007, 176(4):435-444.
2. Susanto O, Trapani J A, Brasacchio D. Controversies in granzyme biology[J]. Tissue Antigens, 2012, 80(6):477-487.
3. Hiebert PR, Granville DJ. Granzyme B in injury, inflammation, and repair[J]. Trends in Molecular Medicine, 2012, 18(12):732-741.
4. Kamata Y, Kimura U, Matsuda H, et al. Relationships among plasma granzyme B level, pruritus and dermatitis in patients with atopic dermatitis[J]. Journal of Dermatological Science, 2016, 84(3).