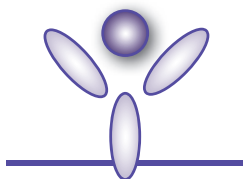


BioLegend®

LEGEND MAX™

ELISA Kit with Pre-coated Plates
ELISA Kit



High Sensitivity Human Granzyme B

Cat. No. 439217

ELISA Kit for Accurate Quantitation of
Human Granzyme B
in Serum, Plasma, and Cell Culture Supernatant

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.



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LEGEND MAX™ High Sensitivity Human Granzyme B ELISA Kit

Introduction:

Granzymes are serine proteases that are released by cytoplasmic granules within cytotoxic T cells and natural killer (NK) cells. Human Granzyme B is one of the five members of the human granzyme family which includes Granzymes A, B, G, H, and K. Granzyme B is expressed as a 32 kD protein encoded by the GZMB gene. While Granzyme B is mostly expressed by CTL (Cytotoxic T Lymphocyte) and NK cells, it is also expressed in resting and activated plasmacytoid dendritic cells. Granzyme B is crucial for the rapid induction of target cell apoptosis by CTL in cell-mediated immune response. Granzyme B and perforin have been shown to induce CTL-mediated target cell DNA fragmentation and apoptosis. Once released from the CTL, Granzyme B binds its receptor, the mannose-6-phosphate/insulin-like growth factor II receptor; and is endocytosed but remains arrested in endocytic vesicles until released by perforin. Once in the cytosol, Granzyme B targets caspase-3 and other caspases directly or indirectly through the mitochondria, initiating the caspase cascade which leads to DNA fragmentation and apoptosis. Elevated Granzyme B levels have been linked with various disease processes.

The LEGEND MAX™ High Sensitivity Human Granzyme B ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with anti-human Granzyme B monoclonal antibody. The detection antibody is a biotinylated anti-human Granzyme B monoclonal antibody. This kit is specifically designed sensitively for the accurate quantitation of human Granzyme B in human serum, plasma, and cell culture supernatant. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume	Part #
Human Granzyme B pre-coated 96-well Strip Microplate	1 plate		750004083
HS Human Granzyme B Detection Antibody	1 bottle	12 mL	750004322
Human Granzyme B Lyophilized Standard	1 vial	Lyophilized	79801
Streptavidin-Polymer HRP	1 bottle	12 mL	750002513
Assay Buffer A	1 bottle	25 mL	78232
Assay Buffer D	1 bottle	25 mL	79383
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
HS Human Granzyme B Detection Antibody	Store opened reagent bottles at 2° - 8°C and use within 1 month
Streptavidin-Polymer HRP	
Assay Buffer A	
Assay Buffer D	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).

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2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin, or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

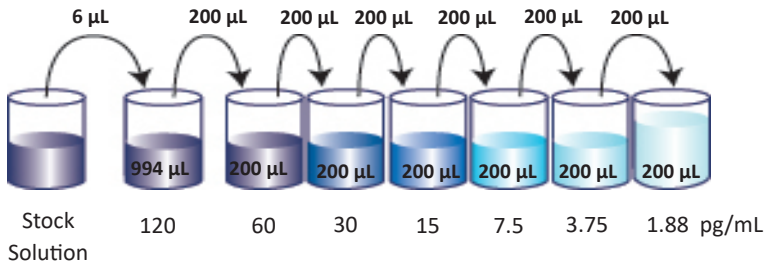
1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human Granzyme B Lyophilized Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/ LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. Serum, EDTA-plasma, Citrate-plasma, and Heparin-plasma samples should be tested initially without any dilution. Cell culture supernatant dilution factor should be determined by the end user; use Assay Buffer A for supernatant dilutions.

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Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 1000 μL of the 120 pg/mL top standard by adding 6 μL of the 20 ng/mL standard stock solution into 994 μL Assay Buffer A. Perform six two-fold serial dilutions of the 120 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human Total Tau standard concentrations in the tubes are 120 pg/mL , 60 pg/mL , 30 pg/mL , 15 pg/mL , 7.5 pg/mL , 3.75 pg/mL and 1.88 pg/mL , respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



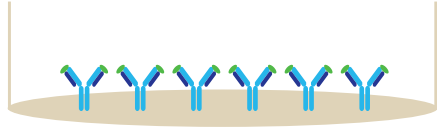
4. Wash the plate 4 times with at least 300 μL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50 μL Assay Buffer A (if testing serum, EDTA-plasma, or citrate-plasma, use Assay Buffer D) into designated well.
6. Add 50 μL of standard dilutions or samples to the appropriate wells.
7. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
9. Add 100 μL of HS Human Granzyme B Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

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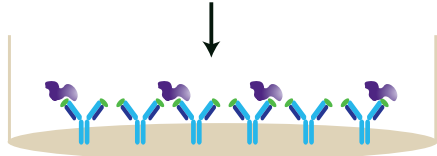
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 μL of Streptavidin-Polymer HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 μL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing Human Granzyme B should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 μL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm and 570 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450.

Assay Procedure Summary

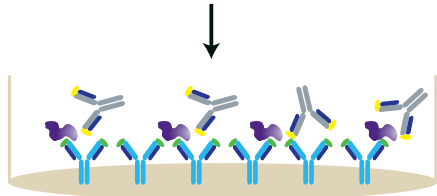
1. Wash 4 times.
Add 50 μ L Assay Buffer A (if testing serum, EDTA-plasma, citrate-plasma, use Assay Buffer D) into each well.



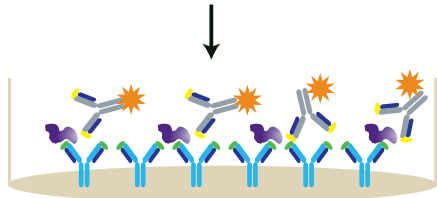
2. Add 50 μ L of standard or sample, incubate 2 hr, RT, shaking



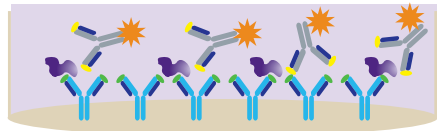
3. Wash 4 times
Add 100 μ L of HS Human Granzyme B Detection Antibody solution.
Incubate 1 hr, RT, shaking



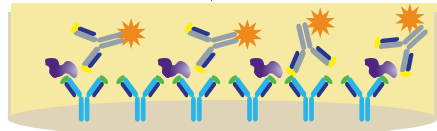
4. Wash 4 times
Add 100 μ L Streptavidin-Polymer HRP solution.
Incubate 30 min, RT, shaking



5. Wash 5 times
Add 100 μ L Substrate Solution F
Incubate 15 min, RT, in the dark



6. Add 100 μ L Stop Solution



7. Read absorbance at 450 nm and 570 nm

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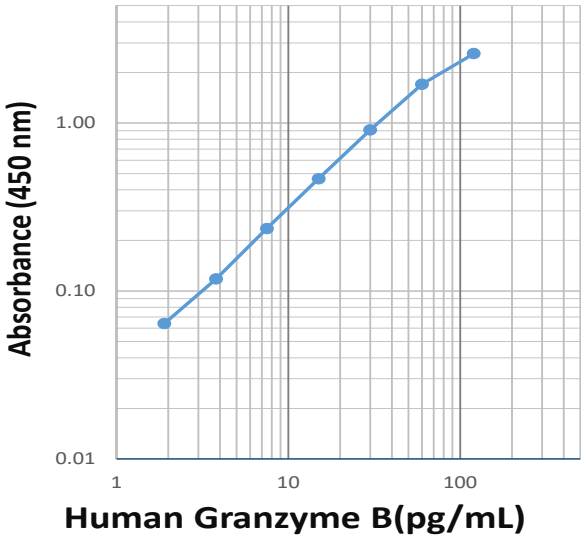
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the analyte concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



LEGEND MAX™ High Sensitivity Human Granzyme B ELISA Kit

Performance Characteristics:

Specificity: This kit recognizes natural and recombinant human Granzyme B. No cross reactivity was observed when this kit was used to analyze the following 34 recombinant proteins at 2.4 ng/mL.

Human	Granzyme A, Perforin, β 2-Microglobulin, CD40L, Cystatin C, Eotaxin, GADPH, G-CSF, GM-CSF, ICAM-1, IP-10, Lipocalin-2, MCP-1, RANTES, sFasL, TIM-1, VCAM-1, CXCL1, PDGF-BB, TGF- α , IL-34, IL-10, IL-8, IL-7, IL-6, IL-5, IL-4, IL-2, IL-1 β .
Mouse	Granzyme B

Sensitivity: The minimum detectable concentration of Human Granzyme B is 0.63 ± 0.20 pg/mL (n=6).

Recovery: Recombinant Human Granzyme B at 3 different concentrations was spiked into human samples: serum, plasma and PBMC (CD3+CD28) cell culture supernatant. Sample recovery was then analyzed with the LEGEND MAX™ High Sensitivity Human Granzyme B kit.

Sample Type	N	% Recovery
Serum	3	77%
EDTA-Plasma	3	94%
Citrate-Plasma	3	83%
Heparin-Plasma	3	83%
PBMC (CD3+CD28) cell culture sup	1	93%

Linearity: Serum, plasma samples were spiked with 70 pg/mL human Granzyme B recombinant protein. No initial dilution was done to PBMC (CD3+CD28) cell culture supernatant. Then, the samples were diluted 2 fold in serial to produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity.

Sample Type	N	% Linearity
Serum	3	111%
EDTA-Plasma	3	110%
Citrate-Plasma	3	107%
Heparin-Plasma	3	110%
PBMC (CD3+CD28) cell culture sup	1	100%

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Intra-Assay Precision: Two samples containing different human Granzyme B concentrations were tested on one plate with 16 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	53.9	10.9
Standard Deviation	0.7	0.2
%CV	1.2%	2.2%

Inter-Assay Precision: Two samples containing different human Granzyme B concentrations were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	55.2	11.9
Standard Deviation	2.0	0.8
%CV	3.7%	6.6%

Biological Samples: Serum, EDTA-plasma, Citrate-plasma, Heparin-plasma samples, and cell culture supernatant (from hu PBMCs stimulated with 3 µg/mL of hu CD3 Ab and 3 µg/mL of hu CD28 Ab for 3 days) were tested for natural human Granzyme B using the LEGEND MAX™ High Sensitivity Human Granzyme B ELISA Kit.

	Serum	EDTA- Plasma	Citrate-Plasma	Heparin-Plasma
N	7	14	6	10
Min (pg/mL)	0.61	1.94	1.58	2.64
Max (pg/mL)	20.7	45.8	16.5	44.8
Mean (pg/mL)	4.4	11.5	4.7	11.9

	hu PBMC (CD3+CD28) cell culture supernatant
N	1
Concentration (pg/mL)	481,706

Troubleshooting Guide:

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Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN ₃)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

BioLegend®

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