Human Granzyme B ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human Granzyme B

Catalog Numbers BMS2027-2 or BMS2027-2TEN

Pub. No. MAN0025556 Rev. A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human Granzyme B ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human Granzyme B.

Summary

Granzymes are exogenous serine proteinases (enzymes) that are released from cytoplasmic granules of cytotoxic lymphocytes (CTLs) and NK cells.

The name *granzymes* is derived from: granules + enzymes. In addition to granzymes, cytoplasmic granules of CTLs and NK cells also contain other proteins, including a pore-forming protein (perforin). Upon binding of the CTL to a target cell (by CTL-receptor and antigen-presenting MHC molecules on the target cell), the contents of the granules are released into the intercellular space. Perforine then binds the target cell membrane, forming transmembrane pores, which allow granzymes to enter the cytosol of the target cell. Granzyme B facilitates cell death through activation of an intracellular caspase cascade. Granzyme A can also induce apoptosis in the target cell, however the molecular mechanisms of the pathway require further investigation.

Percentages of Granzyme A and B positive CTLs can be determined by flow cytometry and immunocytochemical methods for many disorders.

For literature updates, go to **www.thermofisher.com**.

Principles of the test

An anti-human Granzyme B coating antibody is adsorbed onto microwells.

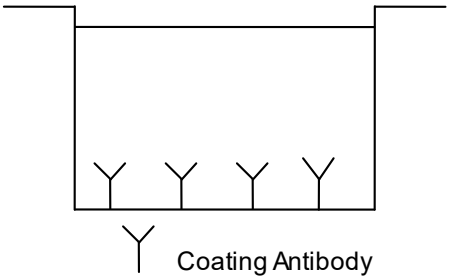

Figure 1 Coated microwell.

Human Granzyme B present in the sample or standard binds to antibodies adsorbed to the microwells.

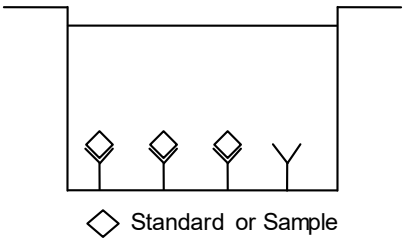

Figure 2 First incubation.

Following incubation unbound biological components are removed during a wash step and a biotin-conjugated anti-human Granzyme B antibody is added and binds to Granzyme B captured by the first antibody.

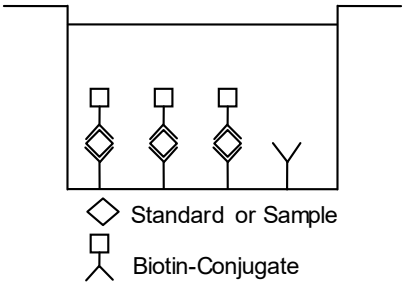

Figure 3 Second incubation.

Following incubation unbound biotin-conjugated anti-human Granzyme B antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human Granzyme B antibody.

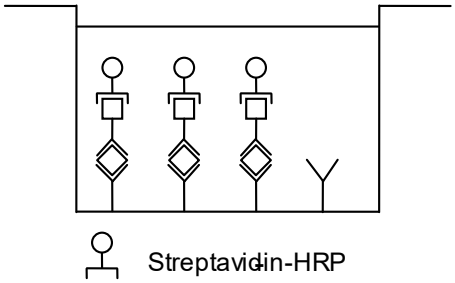

Figure 4 Third incubation.

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

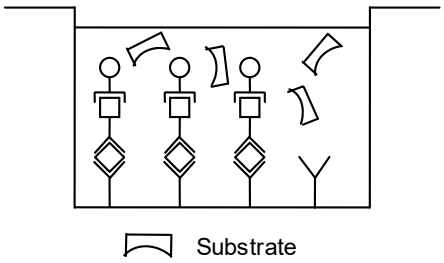

Figure 5 Fourth incubation.

A colored product is formed in proportion to the amount of human Granzyme B present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human Granzyme B standard dilutions and human Granzyme B sample concentration determined.

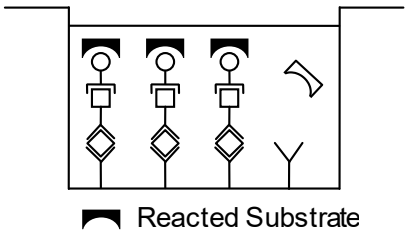

Figure 6 Stop reaction.

Reagents provided

Reagents for human Granzyme B ELISA BMS2027-2 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human Granzyme B

1 vial (70 µL) Biotin-Conjugate anti-human Granzyme B monoclonal antibody

1 vial (150 µL) Streptavidin-HRP

2 vials human Granzyme B Standard lyophilized, 960 pg/mL upon reconstitution

1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

1 bottle (7 mL) Dilution Buffer Concentrate 5x

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

6 Adhesive Films

Reagents for human Granzyme B ELISA BMS2027-2TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human Granzyme B

10 vials (70 µL) Biotin-Conjugate anti-human Granzyme B monoclonal antibody

10 vials (150 µL) Streptavidin-HRP

10 vials human Granzyme B Standard lyophilized, 960 pg/mL upon reconstitution

8 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 bottles (7 mL) Dilution Buffer Concentrate 5x

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

30 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2–8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell culture supernatant and serum were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human Granzyme B. If samples are to be run within 24 hours, they may be stored at 2–8°C.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.

2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1,000 mL graduated cylinder. Bring to final volume of 1,000 mL with glass-distilled or deionized water.

2. Mix gently to avoid foaming.

3. Transfer to a clean wash bottle and store at 2–25°C. The Wash Buffer (1x) is stable for 30 days.

4. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

Dilution buffer (1x)

1. Mix the contents of the bottle well. Add contents of Dilution Buffer Concentrate (5x) (7.0 mL) to 28 mL distilled or deionized water and mix gently to avoid foaming. Store at 2–8°C. The Dilution Buffer is stable for 30 days.

2. Dilution Buffer may be prepared as needed according to the following table:

Number of Strips	Dilution Buffer Concentrate (5x) (mL)	Distilled Water (mL)
1–6	3.5	14.0
1–12	7.0	28.0

Biotin-Conjugate

Note: The Biotin-Conjugate should be used within 30 minutes after dilution.

1. Before opening, spin vial in microcentrifuge to collect reagent at the bottom.

2. Make a 1:200 dilution of the concentrated Biotin-Conjugate solution with Dilution Buffer in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Dilution Buffer (1x) (mL)
1–6	0.03	5.97
1–12	0.06	11.94

Streptavidin-HRP

Note: The Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with Dilution Buffer in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Dilution Buffer (1x) (mL)
1– 6	0.06	5.94
1–12	0.12	11.88

Human Granzyme B standard

1. Reconstitute human Granzyme B standard by addition of distilled water.
2. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 960 pg/mL).
3. Allow the standard to reconstitute for 10–30 minutes. Mix well prior to making dilutions.
4. After usage remaining standard cannot be stored and has to be discarded.
5. Standard dilutions can be prepared directly on the microwell plate (see page 4) or alternatively in tubes (see page 4).

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 1:3 serial dilutions for the standard curve as follows: Pipette 200 µL of Dilution Buffer into tube S1. Pipette 250 µL of Dilution Buffer into tubes S2–S7.
3. Pipette 200 µL of reconstituted standard (concentration = 960 pg/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 480 pg/mL).
4. Pipette 125 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 7 on page 4).

   Dilution Buffer serves as blank.

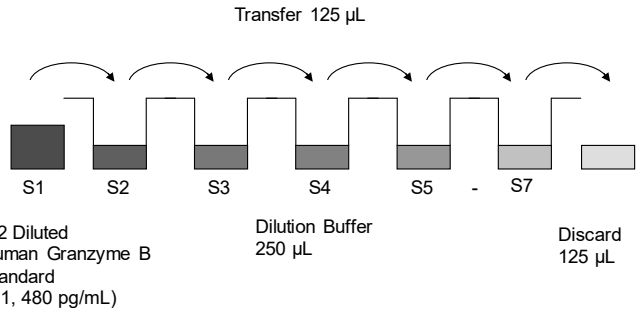

Figure 7 Dilute standards - tubes.

Test protocol

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at –20°C sealed tightly.
2. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
3. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see “External standard dilution” on page 4):

   Add 75 µL of Dilution Buffer in duplicate to standard wells A1, A2. Add 100 µL of Dilution Buffer in duplicate to standard wells B1/2- G1/2. Pipette 75 µL of prepared standard (see Preparation of Standard “Human Granzyme B standard” on page 4, concentration = 960 pg/mL) in duplicate into well A1 and A2 (see Table 1 on page 5). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 480 pg/mL), and transfer 50 µL to wells B1 and B2, respectively (see Figure 8). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human Granzyme B standard dilutions ranging from 480.0 to 0.7 pg/mL. Discard 50 µL of the contents from the last microwells (G1, G2) used.

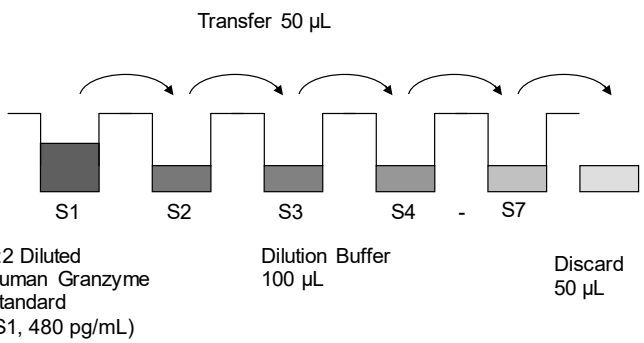

Figure 8 Dilute standards - microwell plate.

In case of an external standard dilution (see "External standard dilution" on page 4), pipette 100 µL of these standard dilutions (S1–S7) in the standard wells according to Table 1 on page 5.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 480.0 pg/mL	Standard 1 480.0 pg/mL	Sample 1	Sample 1
B	Standard 2 160 pg/mL	Standard 2 160 pg/mL	Sample 2	Sample 2
C	Standard 3 53.3 pg/mL	Standard 3 53.3 pg/mL	Sample 3	Sample 3
D	Standard 4 17.8 pg/mL	Standard 4 17.8 pg/mL	Sample 4	Sample 4
E	Standard 5 5.9 pg/mL	Standard 5 5.9 pg/mL	Sample 5	Sample 5
F	Standard 6 2.0 pg/mL	Standard 6 2.0 pg/mL	Sample 6	Sample 6
G	Standard 7 0.7 pg/mL	Standard 7 0.7 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

4. Add 100 µL of Dilution Buffer in duplicate to the blank wells.
5. Add 50 µL of Dilution Buffer to the sample wells.
6. Add 50 µL of each sample in duplicate to the sample wells.
7. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour on a microplate shaker.
8. Prepare Biotin-Conjugate (see page 3).
9. Remove adhesive film and empty wells. Wash microwell strips 5 times according to step 2 of the test protocol. Proceed immediately to the next step.
10. Add 100 µL of Biotin-Conjugate to all wells.
11. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour on a microplate shaker.
12. Prepare Streptavidin-HRP (see page 4).
13. Remove adhesive film and empty wells. Wash microwell strips 5 times according to step 2 of the test protocol. Proceed immediately to the next step.
14. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
15. Cover with an adhesive film and incubate at room temperature (18–25°C) for 30 minutes, if available on a microplate shaker.
16. Remove adhesive film and empty wells. Wash microwell strips 5 times according to step 2 of the test protocol. Proceed immediately to the next step.
17. Pipette 100 µL of TMB Substrate Solution to all wells.
18. Incubate the microwell strips at room temperature (18–25°C) for about 15 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next step of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.
19. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.
20. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human Granzyme B concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human Granzyme B for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human Granzyme B concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Dilution Buffer) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect human Granzyme B levels. Such samples require further external predilution according to expected human Granzyme B values with Dilution Buffer in order to precisely quantitate the actual human Granzyme B level.
- It is suggested that each testing facility establishes a control sample of known human Granzyme B concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 9.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

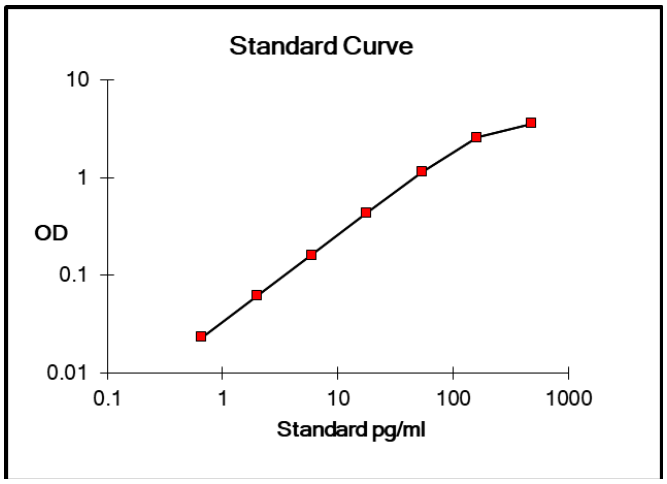

Figure 9 Representative standard curve for Human Granzyme B ELISA Kit. Human granzyme B was diluted in serial 3-fold steps in dilution buffer.

Table 2 Typical data using the Human Granzyme B ELISA Kit (measuring wavelength of 450 nm, reference wavelength of 620 nm).

Standard	Human Granzyme B concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	480.0	3.713 3.731	3.722	0.3
2	160.0	2.701 2.619	2.660	2.2
3	53.3	1.137 1.141	1.139	0.3
4	17.8	0.503 0.53	0.517	3.7
5	5.9	0.279 0.275	0.277	1.1
6	2.0	0.191 0.189	0.190	0.9
7	0.7	0.148 0.157	0.153	4.4
Blank	0.0	0.132 0.131	0.132	0.8

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Because exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks, or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle, and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Performance characteristics

Sensitivity

The limit of detection of human Granzyme B defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.43 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human Granzyme B. Two standard curves were run on each plate. Data below show the mean human Granzyme B concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 6.2%.

Table 3 The mean human Granzyme B concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean concentration (pg/mL)	Coefficient of variation (%)
1	1	283.2	3.2
	2	279.6	3.3
	3	295	6.0
2	1	137.6	3.9
	2	135.9	2.8
	3	146.8	5.2
3	1	63.2	4.1
	2	64.3	3.1
	3	68.4	5.6
4	1	29.8	5.3
	2	32.5	4.0
	3	34.7	2.9
5	1	17.3	6.1
	2	18.3	5.5
	3	18.6	9.0
6	1	7.7	9.7
	2	8.9	8.4
	3	9.7	5.6
7	1	3.3	15.0
	2	4.3	11.4
	3	3.5	10.7

Inter-assay

Assay-to-assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human Granzyme B. Two standard curves were run on each plate. Data below show the mean human Granzyme B concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall inter-assay coefficient of variation was 6.8%.

Table 4 The mean human Granzyme B concentration and the coefficient of variation of each sample.

Sample	Mean concentration (pg/mL)	Coefficient of variation (%)
1	285.9	2.8
2	140.1	4.2
3	65.3	4.2
4	32.3	7.6
5	18	3.6
6	8.8	11.6
7	3.7	13.7

Spike recovery

The spike recovery was evaluated by spiking four levels of human Granzyme B into serum samples. Recoveries were determined in 3 independent experiments with 4 replicates each. The amount of endogenous human Granzyme B in unspiked serum was substracted from the spike values. The overall mean recovery was 97%.

Dilution parallelism

Three serum samples with different levels of human Granzyme B were analyzed at serial 2-fold dilutions with four replicates each. The recovery ranged from 73% to 103% with an overall recovery of 88%.

Sample	Dilution	Expected human Granzyme B concentration (pg/mL)	Observed human Granzyme B concentration (pg/mL)	Recovery of expected human Granzyme B concentration (%)
1	1:4	–	20.1	–
	1:8	10.0	8.4	84
	1:16	4.2	3.4	80
2	1:4	–	132.0	–
	1:8	66.0	66.5	101
	1:16	33.3	34.2	103
3	1:4	–	8.5	–
	1:8	4.2	3.4	80
	1:16	1.6	1.4	81

Sample stability

Freeze-Thaw stability

Aliquots of serum samples were stored at –20°C and thawed three times, and the human Granzyme B levels determined. There was no significant loss of human Granzyme B immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum were stored at –20°C and 2–8°C and the human Granzyme B level determined after 24 hours. There was no significant loss of human Granzyme B immunoreactivity detected during storage under above conditions.

Specificity

The interference of other proteinases was evaluated by spiking these enzymes into a human Granzyme B positive serum. No cross-reactivity was detected, namely not with Proteinase 2 (PR3), Tryptase, Cathepsin G (Cath G), Granzyme A, Human Neutrophil Elastase (HNE), Trypsin, and Chymotrypsin.

Expected values

A panel of 40 sera samples from randomly selected apparently healthy donors (males and females) was tested for human Granzyme B. The detected human Granzyme B levels ranged between 0.1 and 18.5 pg/mL.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

Dilution buffer (1x)

Add Dilution Buffer Concentrate (5x) (7.0 mL) to 28 mL distilled water.

Biotin-Conjugate

Make a 1:200 dilution of Biotin-Conjugate in Dilution Buffer:

Number of Strips	Biotin-Conjugate (mL)	Dilution Buffer (1x) (mL)
1–6	0.03	5.97
1–12	0.06	11.94

Streptavidin-HRP

Make a 1:100 dilution of Streptavidin-HRP in Dilution Buffer:

Number of Strips	Streptavidin-HRP (mL)	Dilution Buffer (1x) (mL)
1–6	0.06	5.94
1–12	0.12	11.88

Human granzyme B standard

Reconstitute lyophilized human Granzyme B standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

Note: If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Dilution Buffer) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 75 µL Dilution Buffer, in duplicate, to standard wells A1, A2. Add 100 µL Dilution Buffer, in duplicate, to standard wells B1/B2 – G1/G2. Pipette 75 µL prepared standard into the first wells and create standard dilutions by transferring 50 µL from well to well. Discard 50 µL from the last wells.

   Alternatively, external standard dilution in tubes (see page 4): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Dilution Buffer, in duplicate, to the blank wells.
5. Add 50 µL Dilution Buffer to sample wells.
6. Add 50 µL sample in duplicate, to designated sample wells.
7. Cover microwell strips and incubate 1 hour at room temperature (18–25°C).
8. Prepare Biotin-Conjugate.
9. Empty and wash microwell strips 5 times with Wash Buffer.
10. Add 100 µL Biotin-Conjugate to all wells.
11. Cover microwell strips and incubate 1 hour at room temperature (18–25°C).
12. Prepare Streptavidin-HRP.
13. Empty and wash microwell strips 5 times with Wash Buffer.
14. Add 100 µL diluted Streptavidin-HRP to all wells.
15. Cover microwell strips and incubate 30 minutes at room temperature (18–25°C).
16. Empty and wash microwell strips 5 times with Wash Buffer.

17. Add 100 µL of TMB Substrate Solution to all wells.

18. Incubate the microwell strips for about 15 minutes at room temperature (18–25°C).

19. Add 100 µL Stop Solution to all wells.

20. Blank microwell reader and measure color intensity at 450 nm.

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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A.0 (30)	7 July 2021	New manual