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

INTRODUCTION

Granzyme B is a serine protease produced mainly by cytotoxic T-lymphocytes and natural killer (NK) cells, and it is stored in their secretory granules. It plays a crucial role in immune defense by inducing apoptosis in virally infected and malignantly transformed cells through a perforin-dependent mechanism. In addition to its cytotoxic function, Granzyme B is involved in extracellular matrix degradation and inflammation, leading to tissue remodeling and chronic inflammation. Elevated levels of Granzyme B have been associated with a variety of diseases, including autoimmune disorders, cardiovascular disease, and impaired wound healing.

Tribioscience's Fast Mouse Granzyme B ELISA is designed to quantitatively detect mouse Granzyme B levels in different tissues including skin, muscle, neural, serum, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate, and fast. The measurement can be finished in 2 hours, with no need for 4-5 hours (Fig. 1). The detection range is from 7 to 500 pg/mL. The levels of mouse Granzyme B samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural mouse Granzyme B protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for mouse Granzyme B was pre-coated onto a microplate. Standards and samples are pipetted into the wells, and then, incubated with HRP-conjugated detection antibody specific for mouse Granzyme B. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is in proportion to the amount of Granzyme B bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

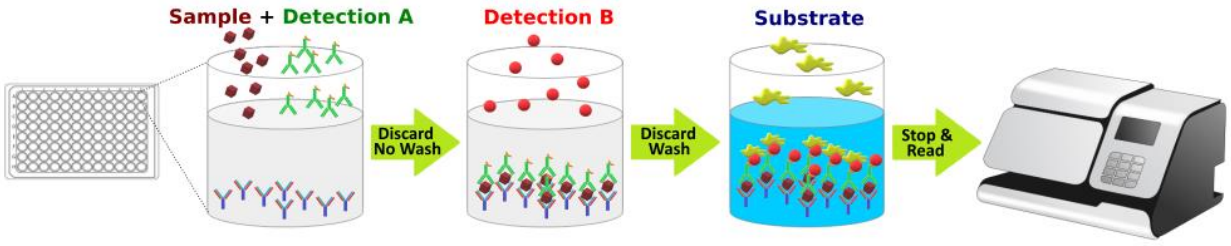


Fig.1: Simple ELISA procedure

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse Granzyme B Microplate	TBS3083A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse Granzyme B.	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Mouse Granzyme B Standard	TBS3083B	30 µL of Recombinant mouse Granzyme B protein (25 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3083C	2.2 mL of Biotin-mouse Granzyme B antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3083D	300 µL of Streptavidin-HRP.	
Assay Diluent	TBS3083E	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10X) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).*

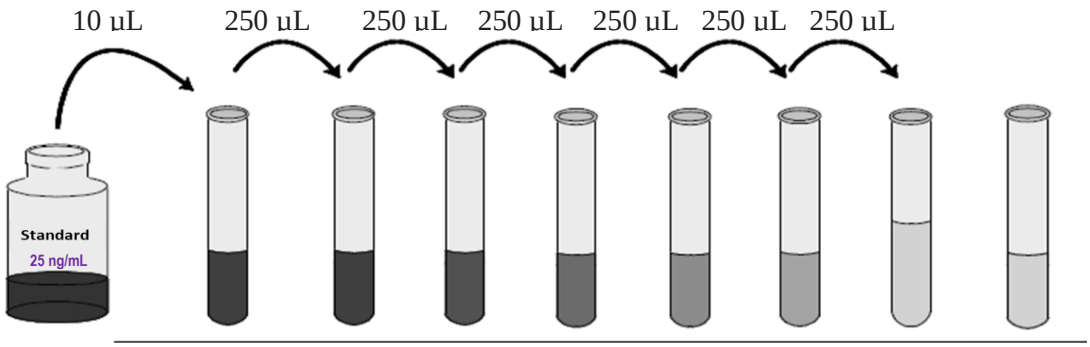
Detection B working solution preparation: Add 240 µL of **Detection B** streptavidin-HRP to 12 mL Assay Diluent (TBS3050E) to prepare Detection B working solution.

Mouse Granzyme B Standard Preparation:

Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as Fig.2 diagram below.**

1. Add 10 µL of the Mouse Granzyme B Standard stock solution (25 ng/mL) by dilution of 50X to tube #1 and mix.
2. Make 2x serial dilutions using the of 500 pg/mL (tube #1) standard solution from tube #2 through #7 with sequential transfer of 250 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 pg/mL. Tube# 8 is blank (0 pg/mL)

Fig.2 Diagram for Mouse Granzyme B standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	490	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	10	250	250	250	250	250	250	0
Final Conc (pg/mL)	500	250	125	62.5	31.25	15.63	7.81	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours with shaking**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B working solution** to each well. Incubate at **RT for 1 hour with shaking.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20 minutes with shaking** (*Protect from light*). The color becomes blue.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse Granzyme B concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve (R^2=0.9998) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

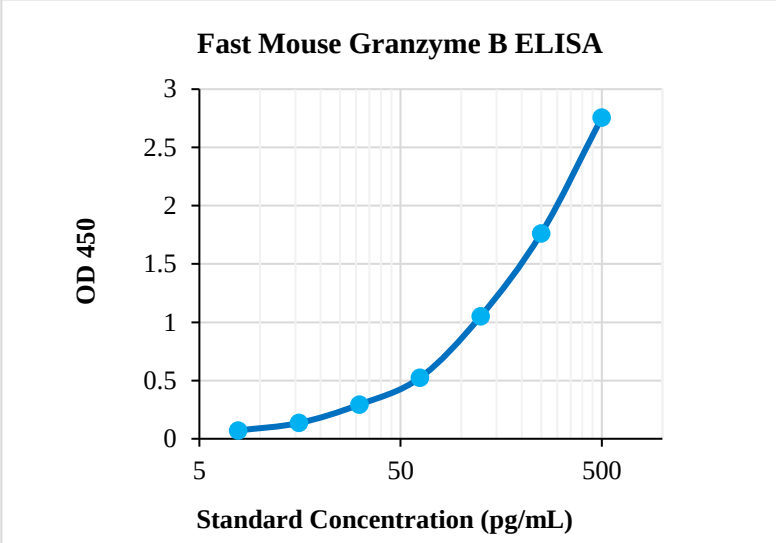
The minimum detectable dose (MOD) of mouse Granzyme B is typically 13 pg/ml.
The Intra-assay CV is 3.79% the Inter-assay CV is <10%.

SPECIFICITY

This assay recognizes natural and recombinant mouse Granzyme B.

RELATIVE PRODUCTS

TBS3030	Fast Mouse IL-1β ELISA
TBS3031	Fast Mouse IL-2 ELISA
TBS3032	Fast Mouse IL-4 ELISA
TBS3040	Fast Mouse IL-6 ELISA
TBS3044	Fast Mouse IL-10 ELISA
TBS3047	Fast Mouse IL-12 p70 ELISA
TBS3049	Fast Mouse IL-13 ELISA
TBS3060	Fast Mouse KC ELISA
TBS3070	Fast Mouse NGF ELISA
TBS3079	Fast Mouse GM-CSF ELISA
TBS3080	Fast Mouse G-CSF ELISA
TBS3084	Fast Mouse IFN-γ ELISA
TBS3085	Fast Mouse TGF ELISA
TBS3086	Fast Mouse MCPT-1 ELISA
TBS3090	Fast Mouse IL-17AF ELISA
TBS3091	Fast Mouse IL-19 ELISA
TBS3092	Fast Mouse IL-21 ELISA
TBS3093	Fast Mouse IL-22 ELISA
TBS3094	Fast Mouse IL-23 ELISA
TBS3095	Fast Mouse IL-27 ELISA
TBS3096	Fast Mouse IL-28B ELISA
TBS3097	Fast Mouse IL-33 ELISA



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