



EAGLE  
BIOSCIENCES

# Mouse Granzyme B ELISA Assay Kit

Catalog Number:

**GZM11-K01 (1 x 96 wells)**

*For Research Use Only. Not for use in diagnostic procedures.*

v. 1.0 (26 AUG 24)

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## INTENDED USE

The Eagle Biosciences Mouse Granzyme B ELISA Assay Kit is intended for the quantitative measurement of Granzyme B in mouse serum/plasma, urine, and more. The Mouse Granzyme B ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## REAGENTS PROVIDED

Content	Volume
CP (Coated Plate)	96 well
S (Standard)	9 vial
DA (Detect Antibody)	6 mL/bottle
SH (Streptavidin-HRP)	12 mL/bottle
AB (Assay Buffer 1 x)	12 mL/bottle
TS (TMB Substrate)	12 mL/bottle
SS (Stop Solution)	12 mL/bottle
WB (Wash Buffer 10x)	50 mL/bottle
SF (Sealer Film)	6 pieces

Note: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

## REAGENT PREPARATION

### Washing Buffer (1x) Preparation:

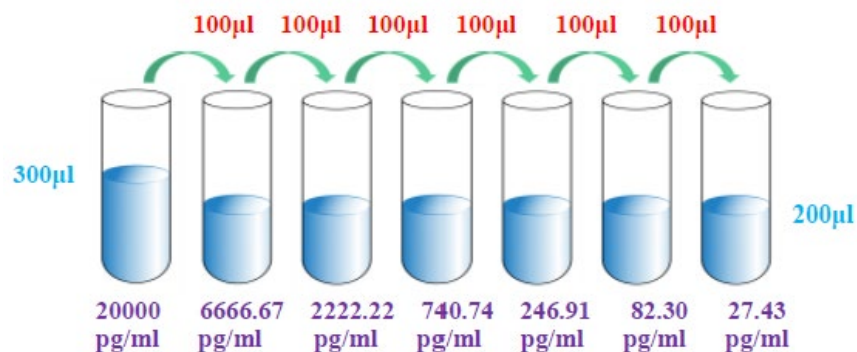
Pour entire contents (50 mL) of the **Washing Buffer Concentrate** (10x) into a clean 500 mL graduated cylinder. Bring to final volume of 500 mL with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25°C.

### Standard Curve Preparation:

S1 to S7 and S0 is ready to use for serum and plasma.

For other sample types, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. For urine samples use AB (Assay Buffer) to prepare the standard curve.

The Mouse Granzyme B Standard 200,000 pg/mL 30  $\mu$ L + 270  $\mu$ L SPB serves as the high standard (20,000 pg/mL). Pipette 200  $\mu$ L of SPB into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/mL).





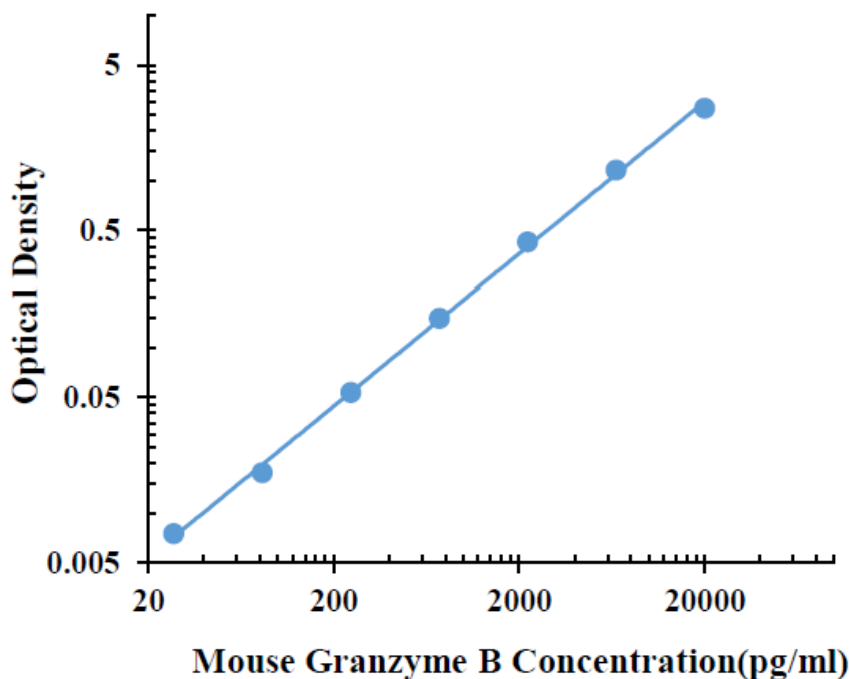
## ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess CP (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50  $\mu\text{L}$  of AB (Assay Buffer) to each well.
4. Add 10  $\mu\text{L}$  of Standard or Sample per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50  $\mu\text{L}$  of DA (Detect Antibody) to each well.
6. Cover with an SF (Sealer Film). Incubate at room temperature (18 to 25°C) for 1 hour on a microplate shaker set to 500 rpm.
7. Aspirate each well and wash, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300  $\mu\text{L}$ ). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 100  $\mu\text{L}$  of SH (Streptavidin-HRP) to each well.
9. Cover with a new SF (Sealer Film). Incubate at room temperature (18 to 25°C) for 30 minutes on a microplate shaker set at 500 rpm.
10. Repeat aspiration/wash as in step 7.
11. Add 100  $\mu\text{L}$  of TS (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
12. Add 100  $\mu\text{L}$  of SS (Stop Solution) to each well.
13. Determine the optical density within 30 minutes, using a microplate reader set to 450 nm corrected with 570 nm or 630 nm.

## TYPICAL DATA

### Mouse Granzyme B Typical Standard





pg/mL	O.D.	Average	Corrected
0.00	0.0150	0.0130	0.0140
27.43	0.0230	0.0200	0.0215
82.30	0.0340	0.0290	0.0315
246.91	0.0680	0.0670	0.0675
740.74	0.1650	0.1630	0.1640
2222.22	0.4570	0.4220	0.4395
6666.67	1.2100	1.1460	1.1780
20000.00	2.7790	2.7510	2.7650

### SENSITIVITY

The minimum detectable dose (MDD) of Granzyme B is typically less than 9.91 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

### PRECISION

#### Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

#### Inter-assay Precision (Precision between assays)

Sample Number	Intra-assay Precision			Inter-assay Precision		
	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average (ng/mL)	341.6	1646.5	5204.5	367.9	1876.2	5851.4
Standard Deviation	22.6	94.6	295.4	8.6	63.2	192.3
Coefficient of variation (%)	6.6	5.7	5.7	2.3	3.4	3.3

### RECOVERY

The spike recovery was evaluated by spiking 3 levels of Mouse Granzyme B into healthy mouse serum samples. The un-spiked serum was used as a blank in this experiment. The recovery ranged from 107% to 132% with an overall mean recovery of 116%.

### LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of Granzyme B into mouse serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay. The linearity ranged from 94% to 111% with an overall mean recovery of 103%.

### SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy mice were evaluated for the presence of Granzyme B in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (pg/mL)	Detectable %	Mean of Detectable (pg/mL)
Serum	30	10.64 - 35.31	92%	24.13

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.



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