



Gasdermin D (Mouse) ELISA Kit

07/20

(Catalog # E4848-100; 96 assays, Storage at 4°C)

I. Introduction:

The gasdermin family members contain N-terminal domains that are capable of forming membrane pores to induce cytolysis, whereas the C-terminal domains of gasdermins function as inhibitors of such cytolysis through intramolecular domain association. Caspase-1 or -11 cleavage of gasdermin D is required for regulation of pyroptosis: upon protease cleavage of the gasdermin N- and C-domain linker, the disruption of the intramolecular domain interaction in the presence of lipids releases the N-domain to assemble oligomeric membrane pores that trigger cell death. Gasdermin D seems to be a key effector in the LPS-induced lethal sepsis. BioVision's Gasdermin D (Mouse) ELISA Kit is based on the Sandwich-ELISA principle. An antibody specific for Gasdermin D has been precoated onto the 96-well microtiter plate. Standards (STD) and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, Gasdermin D is recognized by the addition of a detection antibody specific for Gasdermin D (C-terminus) (DET). After removal of excess antibody, HRP conjugated anti-Guinea Pig IgG (HRP) is added. Following a final washing, peroxidase activity is quantified using the TMB substrate. The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of mouse Gasdermin D in the samples.

II. Applications:

in vitro quantitative determination of Gasdermin D concentrations in cell culture supernatants and cell extracts.

Sensitivity: 14 pg/ml

Detection Range: 15.625 pg/ml - 1000 pg/ml

Specificity: This ELISA is specific for the measurement of natural and recombinant mouse Gasdermin D (fulllength and C-terminus cleaved fragment). It does not detect human Gasdermin D.

III. Sample Type:

Culture supernatants and cell extracts

IV. Kit Contents:

Components	E4848-100	Part Number
Micro ELISA Plate	6 x 16-well strips	E4848-100-1
Wash Buffer (10x)	2 x 30 ml	E4848-100-2
ELISA Buffer (10x)	2 x 30 ml	E4848-100-3
Detection Antibody	30 μΙ	E4848-100-4
HRP (HRP-conjugated anti-guinea pig IgG) (100x)	150 µl	E4848-100-5
Gasdermin D Standard (lyophilized)	100 ng	E4848-100-6
TMB Substrate Solution	12 ml	E4848-100-7
Stop Solution	12 ml	E4848-100-8
Plate Sealer	2	E4848-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

Store at 4°C. After standard reconstitution, prepare aliquots and store at -20°C. Avoid freeze/thaw cycles.

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- Wash Buffer (10x): Dilute with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10x + 270 ml water) to obtain Wash Buffer 1x.
- ELISA Buffer (10x): Dilute with deionized water 1:10 before use (e.g. 10 ml ELISA Buffer 10x + 90 ml water) to obtain ELISA Buffer 1x.
- Detection Antibody: Dilute to 1:500 in ELISA Buffer 1x (20 µl Detection Antibody + 10 ml ELISA Buffer 1X). NOTE: The diluted Detection Antibody is not stable and cannot be stored!
- HRP Conjugated anti-guinea pig IgG (100x): Dilute to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1x (1:100). **NOTE:** The diluted HRP is used within one hour of preparation.
- Standard: Reconstitute with 100 µl of ELISA Buffer 1x. This reconstitution produces a stock solution of 1 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes at room temperature. Mix well prior to making dilutions. NOTE: The reconstituted standard is aliquoted and stored at -20°C! Dilute the standard protein concentrate (STD) (1 µg/ml) in ELISA Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in ELISA Buffer 1X is recommended. Suggested standard points are: 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 0 pg/ml.

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• Add10 µl of 1 µg/ml of Gasdermin D standard to 990 µl of 1x ELISA Buffer to prepare 10 ng/ml stock solution. Add 100 µl of 10 ng/ml Gasdermin D standard to 900 µl of 1x ELISA Buffer to prepare 1000 pg/ml standard. Prepare 6 tubes; add 300 µl of 1x ELISA Buffer to each tube. Pipette 300 µl of the 1000 pg/ml stock solution to the first tube and mix up to produce a 500 pg/ml standard. Transfer 300 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay. After standard reconstitution, prepare aliquots and store at -20°C.

VIII. Sample Preparation:

- Cell Culture Supernatants: Dilute in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.

 NOTE: As a starting point, undiluted or 1/2 dilution of cell culture supernatants is recommended! If sample values fall outside the detection range of the assay, a higher dilution may be required!
- Cell extracts: (lysed in Triton X-100 based buffer) have to be diluted in ELISA Buffer 1x. Samples containing visible precipitates must be clarified before use.

 NOTE: As a starting point, 1/4 dilution of cell extracts is recommended! If sample values fall outside the detection range of the assay, a

IX. Assay Protocol:

lower or higher dilution may be required!

<u>Note:</u> Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

- 1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel minibags and stored at 4°C.
 - **Note:** Remaining 16-well strips coated with Gasdermin D antibody when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.
- 2. Add 100 µl of the different standards and samples into the appropriate wells in duplicate.
- 3. Cover the plate with plastic film and incubate for 2 hours at room temperature.
- 4. Aspirate the coated wells and add 300 μl of 1x Wash Buffer using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
- 5. Add 100 µl to each well of the diluted **Detection Antibody**.
- 6. Cover the plate with plastic film and incubate for 1 hour at room temperature.
- 7. Aspirate the coated wells and add 300 µl of 1x Wash Buffer using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
- 8. Add 100 µl to each well of the diluted HRP-conjugated anti-guinea pig IgG (HRP)
- 9. Cover the plate with plastic film and incubate for 30 minutes at room temperature.
- 10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or autowasher. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
- 11. Add 100 µl to each well of TMB substrate solution.
- **12.** Allow the color reaction to develop at room temperature in the dark for 10-15 minutes. Do not cover the plate.
- **13.** Stop the reaction by adding 100 µl of **Stop Solution**. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
- 14. Measure the OD at 450 nm in an ELISA reader.

Optical Density (mean)	
2.028	
1.209	
0.669	
0.339	
0.171	
0.102	
0.052	
0	

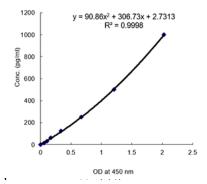
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X. Calculation:

- Average the duplicate readings for each standard and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding Gasdermin D concentration (ng/ml) on the vertical axis
- Calculate the mouse Gasdermin D concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate the concentration of mouse Gasdermin D in the sample.

XI. Precision:

Intra-assay Precision: Four samples of known concentrations of mouse Gasdermin D were assayed in replicates 8 times to test precision within an assay.





Samples	Means (ng/ml)	SD	CV (%)	n
A1	99.60	4.51	4.53	6
A2	9.96	0.98	9.79	6
A3	74.16	5.91	7.96	6
A4	51.12	3.32	6.49	6

Inter-assay Precision: Four samples of known concentrations of mouse Gasdermin D were assayed in 5 separate assays to test precision between assays.

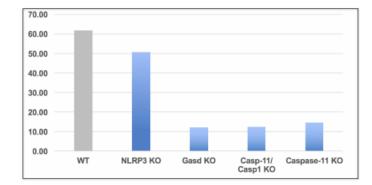
Samples	Means (pg/ml)	SD	CV (%)	n
B1	96.17	4.69	4.88	5
B2	50.18	3.42	6.82	5
B3	22.77	1.66	7.28	5
B4	47.75	3.93	8.24	5

XII. Specificity:

This ELISA is specific for the measurement of natural and recombinant mouse Gasdermin D (full-length and C-terminus cleaved fragment). It does not detect human Gasdermin D.

Gasdermin D is tested from supernatants of Bone Marrow-Derived Macrophages cells (BMDMs) transfected with LPS from different knockout mice strains (see figure 1). Only the supernatants from WT and NLRP3-/- strains contain the protein Gasdermin D. Gasdermin D is also tested from cell extracts (lysed with a Triton X-100 buffer) of Bone Marrow-Derived Macrophages cells from WT and Gasdermin D knockout mice strains (Figure 2). Both figures confirm the specificity of the Gasdermin (mouse) ELISA Kit.





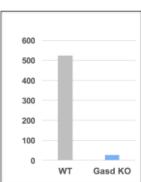


Figure (A): Detection of Gasdermin D in supernatants of BMDMs transfected with LPS from different strains (B) Detection of Gasdermin D in cell extracts of BMDMs from WT and Gasdermin D KO mice strains

XIII. RELATED PRODUCTS:

- IL-1β (rat) ELISA Kit (K4796)
- IL-1β (Human) ELISA Kit (E4818)
- IL-18 (Human) ELISA Kit (K4227)
- Caspase-1 (Mouse) ELISA Kit (K4180)

FOR RESEARCH USE ONLY! Not to be used on humans.