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Research
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Mouse Gasdermin D ELISA

Product Data Sheet

Cat. No.: RAG025R

For Research Use Only

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**»» This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.**

»» Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The Mouse Gasdermin D ELISA Kit is to be used for the in vitro quantitative determination of mouse Gasdermin D in cell culture supernatants and cell extracts. This ELISA Kit is for research use only.

2. HANDLING, STORAGE

- Reagent must be stored at 2-8°C when not in use.
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

3. INTRODUCTION

Inflammasomes are multimeric protein complexes that comprise a sensor (e.g. NLRP3), an adaptor (ASC /Pycard) and a protease (procaspase-1) (1). An inflammasome assembles in response to a diverse range of pathogen-associated or danger-associated molecular patterns (PAMPs or DAMPs), or perturbations in cytoplasmic homeostasis (term 'homeostasis-altering molecular processes' (HAMPs))(2). The inflammasome platform leads to activation of caspase-1, which further induces maturation of interleukins 1 β and 18 (IL-1 β and IL-18) through proteolytic cleavage of pro-IL-1 β and pro-IL-18. Activated caspase-1, and also the recently characterized caspase-11 non-canonical inflammasome pathway, cleave the newly discovered intracellular protein Gasdermin D (3,4). The Gasdermin family members contain N-terminal domains that are capable of forming membrane pores, 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 caspase 1/11 cleavage of the Gasdermin N- and C-domain linker, the cleaved N-terminal fragment of Gasdermin D oligomerizes and forms pores on the host cell membrane (5), leading to a cell death called pyroptosis and further activation of inflammasomes by triggering K⁺ efflux (6). Gasdermin D forming pores regulate the non-conventional secretion of cytokines such as IL-1 β , in response to cytosolic LPS and other activators of the inflammasome (7). Neutrophil extrusion of neutrophil extracellular traps (NETs) and concomitant cell death (NETosis), a particular neutrophil defense against pathogens, are dependent on Gasdermin D (8). Gasdermin D-mediated pyroptosis is regulated at the level of lipid peroxidation (9) and seems to be a key effector in the LPS-induced lethal sepsis (10).

4. TEST PRINCIPLE

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of mouse Gasdermin D in cell culture supernatants and in cell extracts. 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 substrate 3,3',5,5'-tetramethylbenzidine (TMB). 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.

5. TECHNICAL HINTS

- It is recommended that all standards and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 μ l should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep Substrate Solution protected from light.
- The Stop Solution consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

6. REAGENT SUPPLIED

Kit Components	Quantity
1 plate coated with mouse Gasdermin D Antibody	6x16-well strips
2 bottles Wash Buffer 10X	30 ml
2 bottle ELISA Buffer 10X	30 ml
1 vial Detection Antibody	30 μ l
1 vial HRP 100x (HRP-conjugated anti-guinea pig IgG)	150 μ l
1 vial mouse Gasdermin D Standard (lyophilized)	100 ng
1 bottle TMB Substrate Solution	12 ml
1 bottle Stop Solution	12 ml
2 plate sealers (plastic film)	
2 silica Gel Minibags	

7. MATERIALS REQUIRED BUT NOT SUPPLIED

- Microtiterplate reader at 450 nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. PREPARATION OF REAGENTS

NOTE: Prepare just the appropriate amount of the buffers necessary for the assay.

- **Wash Buffer 10X** has to be diluted 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** has to be diluted with deionized water 1:10 before use (e.g. 10 ml ELISA Buffer 10X + 90 ml water) to obtain ELISA Buffer 1X.

- **Detection Antibody (DET)** has to be diluted to 1:500 in ELISA Buffer 1X (20 µl DET + 10 ml ELISA Buffer 1X).

NOTE: *The diluted Detection Antibody is not stable and cannot be stored!*

- **HRP 100x (HRP-conjugated anti-guinea pig IgG)** has to be diluted 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.*

- **Mouse Gasdermin D Standard (STD)** has to be reconstituted 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.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
10 ng/ml	10µl of Gasdermin D (STD) (1 µg/ml)	990 µl of ELISA Buffer 1X

Dilute further for the standard curve:

To obtain	Add	Into
1000 pg/ml	100µl of Gasdermin D (10 ng /ml)	900 µl of ELISA Buffer 1X
500 pg/ml	300 µl of Gasdermin D (1000 pg/ml)	300 µl of ELISA Buffer 1X
250 pg/ml	300 µl of Gasdermin D (500 pg/ml)	300 µl of ELISA Buffer 1X
125 pg/ml	300 µl of Gasdermin D (250 pg/ml)	300 µl of ELISA Buffer 1X
62.5 pg/ml	300 µl of Gasdermin D (125 pg/ml)	300 µl of ELISA Buffer 1X
31.25 pg/ml	300 µl of Gasdermin D (62.5 pg/ml)	300 µl of ELISA Buffer 1X
15.625 pg/ml	300 µl of Gasdermin D (31.25 pg/ml)	300 µl of ELISA Buffer 1X
0 pg/ml	300 µl of ELISA Buffer 1X	Empty tube

9. PREPARATION OF SAMPLES

Cell Culture Supernatants have to be diluted 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 lower or higher dilution may be required!

10. ASSAY PROCEDURE

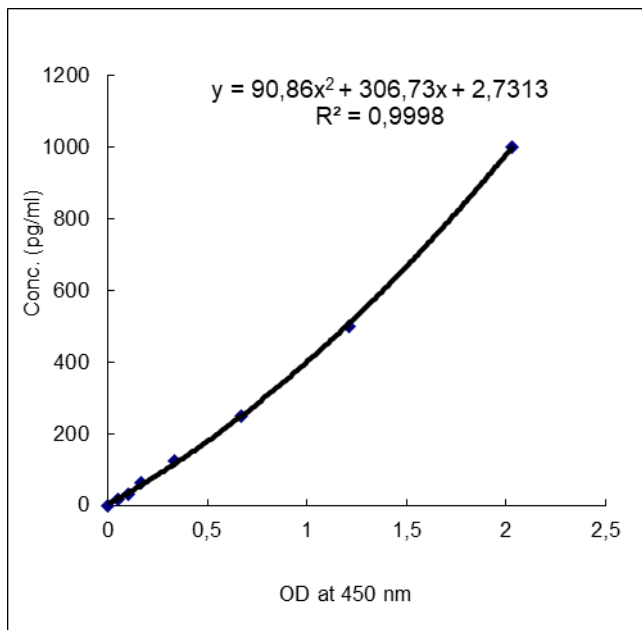
<input type="checkbox"/>	1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel minibags and stored at 4°C. NOTE: <i>Remaining 16-well strips coated with Gasdermin D antibody when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.</i>
<input type="checkbox"/>	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of cell culture supernatant or cell extract samples in duplicate to the wells (see 8.1. Preparation and Storage of Reagents and 8.2 Preparation of Samples).
<input type="checkbox"/>	3. Cover the plate with plastic film and incubate for 2 hours at room temperature .
<input type="checkbox"/>	4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X 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.
<input type="checkbox"/>	5. Add 100 µl to each well of the diluted Detection Antibody (DET) (see 8.1 Preparation and Storage of Reagents).
<input type="checkbox"/>	6. Cover the plate with plastic film and incubate for 1 hour at room temperature .
<input type="checkbox"/>	7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X 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.
<input type="checkbox"/>	8. Add 100 µl to each well of the diluted HRP-conjugated anti-guinea pig IgG (HRP) (see 8.1. Preparation and Storage of Reagents).
<input type="checkbox"/>	9. Cover the plate with plastic film and incubate for 30 minutes at room temperature .
<input type="checkbox"/>	10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X 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.
<input type="checkbox"/>	11. Add 100 µl to each well of TMB substrate solution (TMB).
<input type="checkbox"/>	12. Allow the color reaction to develop at room temperature in the dark for 10-15 minutes . Do not cover the plate.
<input type="checkbox"/>	13. Stop the reaction by adding 100 µl of Stop Solution (STOP). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (STOP) is added.
	! CAUTION: STOP SOLUTION IS A CORROSIVE SOLUTION!
<input type="checkbox"/>	14. Measure the OD at 450 nm in an ELISA reader.

11. CALCULATIONS

- 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 (see TYPICAL DATA).
- 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.

The following data are obtained using the different concentrations of standard as described in this protocol:

Figure: Standard curve



Standard Gasdermin D (pg/ml)	Optical Density (mean)
1000	2.028
500	1.209
250	0.669
125	0.339
62.5	0.171
31.25	0.102
15.625	0.052
0	0

12. PERFORMANCE CHARACTERISTICS

- **Sensitivity** (Limit of detection)

The lowest level of mouse Gasdermin D that can be detected by this assay is 14 pg/ml.

NOTE: The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard.

- **Assay Range**

15.625 pg/ml – 1000 pg/ml

- **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.

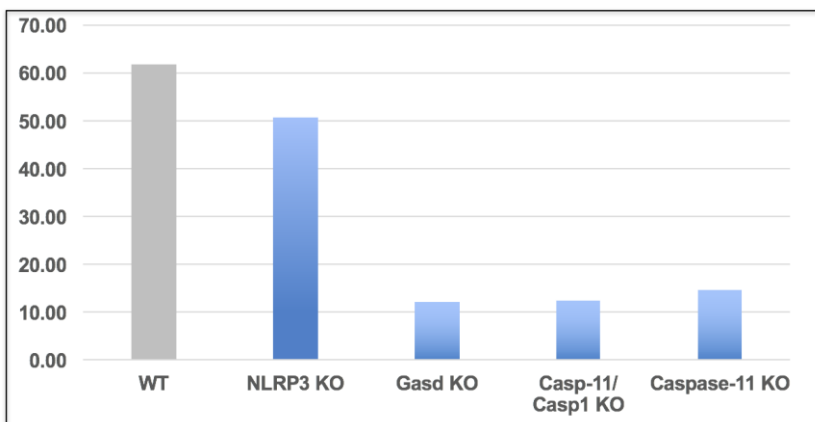


Figure 1: Detection of Gasdermin D in supernatants of BMDMs transfected with LPS from different strains

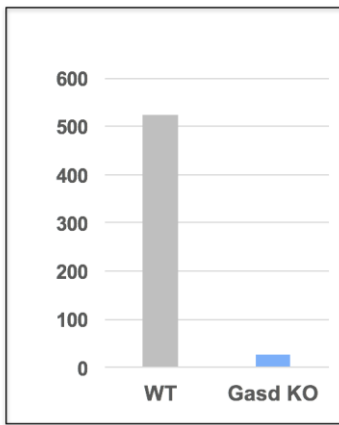


Figure 2: Detection of Gasdermin D in cell extracts of BMDMs from WT and Gasdermin D KO mice strains

- **Precision:**

Intra-assay:

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

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

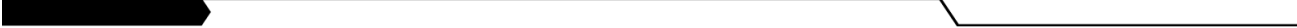
13. TROUBLESHOOTING

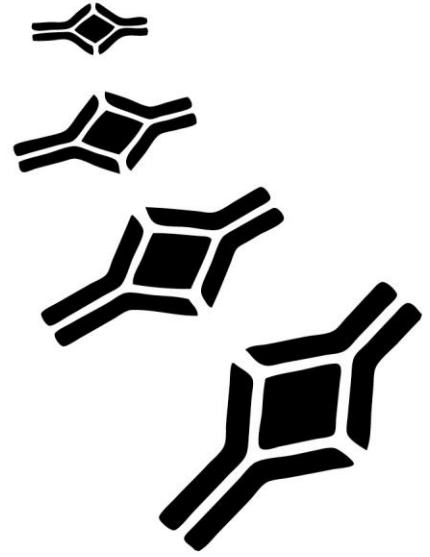
PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. REFERENCES

1. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta: F. Martinon, et al.; *Mol. Cell* 10, 417 (2002)
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8. Noncanonical inflammasome signaling elicits gasdermin D-dependent neutrophil extracellular traps: K.W. Chen, et al.; *Sci. Immunol.* 3, 26 (2018)
9. Lipid Peroxidation Drives Gasdermin D-mediated Pyroptosis in Lethal Polymicrobial Sepsis: R. Kang, et al.; *Cell Host Microbe* 24, 97 (2018)
10. Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis: J.K. Rathkey, et al.; *Sci. Immunol.* 3, 26 (2018)

NOTES





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