Product Information & ELISA Manual

Mouse GSDMDC1 ELISA Kit (Colorimetric)
NBP3-43460

Enzyme-linked Immunosorbent Assay
for quantitative detection.

Contact

Web: www.bio-techne.com/brands/novus-biologicals/
Email: nb-customerservice@bio-techne.com
P: 888.506.6887 // P: 303.730.1950 // F: 303.730.1966

Novus kits are guaranteed for 6 months from date of receipt.

Table of Contents

1. Intended Use

The Mouse GSDMDC1 ELISA Kit (Colorimetric) is to be used for the *in vitro* quantitative determination of mouse GSDMDC1 in cell culture supernatants and cell extracts. This ELISA Kit is for research use only.

2. 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 1β and 18 (IL-1β and IL-18) through proteolytic cleavage of pro-IL-1β and pro-IL-18. Activated caspase-1, and also the recently characterized caspase-11 non-canonical inflammasome pathway, cleave the newly discovered intracellular protein GSDMDC1 (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 GSDMDC1 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 GSDMDC1 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). GSDMDC1 forming pores regulate the non-conventional secretion of cytokines such as IL-1β, 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 GSDMDC1 (8). GSDMDC1-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).

3. General 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)

(2) Homeostasis-altering molecular processes as mechanisms of inflammasome activation: A. Liston & S.L. Masters; Nat. Rev. Immunol. **17**, 208 (2017)

(3) Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling: N Kayagaki, et al.; Nature **526**, 666 (2015)

(4) Mechanisms of Gasdermin Family Members in Inflammasome Signaling and Cell Death: S. Feng, et al.; J. Mol. Biol. **430**, 3068 (2018)

(5) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores: X. Liu, et al.; Nature **535**, 153 (2016)

(6) GSDMDC1 Restrains Type I Interferon Response to Cytosolic DNA by Disrupting Ionic Homeostasis: I. Banerjee**,** et al.; Immunity 49, 413 (2018)

(7) The Pore-Forming Protein GSDMDC1 Regulates Interleukin-1 Secretion from Living Macrophages: C.L. Evavold, et al.; Immunity **48**, 35 (2018)

(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 GSDMDC1-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)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of mouse GSDMDC1 in cell culture supernatants and in cell extracts. An antibody specific for GSDMDC1 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, GSDMDC1 is recognized by the addition of a detection antibody specific for GSDMDC1 (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 GSDMDC1 in the samples.

5. 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.

6. Kit Components

1 plate coated with mouse GSDMDC1 Antibody	(6 x 16-well strips)	
2 bottles Wash Buffer 10X	(2 x 30 ml)	(Wash Buffer 10X)
2 bottles ELISA Buffer 10X	(2 x 30 ml)	(ELISA Buffer 10X)
1 vial Detection Antibody	(30 µl)	(DET)
1 vial HRP 100X (HRP-conjugated anti-guinea pig IgG)	(150 µl)	(HRP 100X)
1 vial mouse GSDMDC1 Standard (lyophilized)	(100 ng)	(STD)
1 bottle TMB Substrate Solution	(12 ml)	(TMB)
1 bottle Stop Solution	(12 ml)	(STOP)
2 plate sealers (plastic film)		
2 silica Gel Minibags		

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450nm
- 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. General ELISA Protocol

8.1. Preparation and Storage 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 GSDMDC1 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.

Dilute for the standard curve:

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
10 ng/ml	10µl of GSDMDC1 (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 GSDMDC1 (10 ng /ml)	900 µl of ELISA Buffer 1X
500 pg/ml	300 µl of GSDMDC1 (1000 pg/ml)	300 µl of ELISA Buffer 1X
250 pg/ml	300 µl of GSDMDC1 (500 pg/ml)	300 µl of ELISA Buffer 1X
125 pg/ml	300 µl of GSDMDC1 (250 pg/ml)	300 µl of ELISA Buffer 1X
62.5 pg/ml	300 µl of GSDMDC1 (125 pg/ml)	300 µl of ELISA Buffer 1X
31.25 pg/ml	300 µl of GSDMDC1 (62.5 pg/ml)	300 µl of ELISA Buffer 1X
15.625 pg/ml	300 µl of GSDMDC1 (31.25 pg/ml)	300 µl of ELISA Buffer 1X
0 pg/ml	300 µl of ELISA Buffer 1X	Empty tube

8.2. Sample collection, storage and dilution

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!*

8.3. Assay Procedure (Checklist)

☐	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 GSDMDC1 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 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).**
☐	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 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.
☐	5. Add 100 µl to each well of the diluted Detection Antibody **(DET) (see 8.1 Preparation and Storage of Reagents)**.
☐	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 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.
☐	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)**.
☐	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 auto-washer. 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 **(TMB)**.
☐	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 (**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!
☐	14. Measure the OD at 450 nm in an ELISA reader.

9. Calculation of Results

- 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 GSDMDC1 concentration (ng/ml) on the vertical axis (see **10.** TYPICAL DATA).
- Calculate the mouse GSDMDC1 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 GSDMDC1 in the sample.

10. Typical Data

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

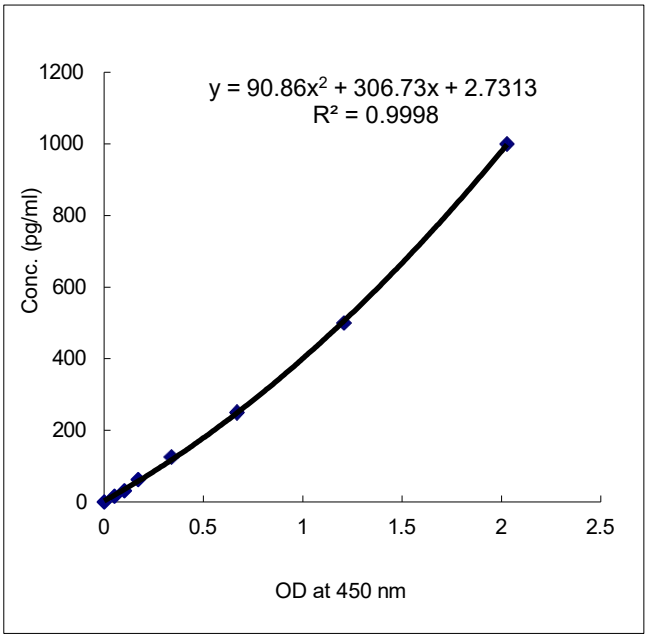

Figure: Standard curve

Standard GSDMDC1 (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

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of mouse GSDMDC1 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.*

B. Assay range: 15.625 pg/ml – 1000 pg/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant mouse GSDMDC1 (full-length and C-terminus cleaved fragment). It does not detect human GSDMDC1.

GSDMDC1 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 GSDMDC1. GSDMDC1 is also tested from cell extracts (lysed with a Triton X-100 buffer) of Bone Marrow-Derived Macrophages cells from WT and GSDMDC1 knockout mice strains (Figure 2). Both figures confirm the specificity of the Gasdermin (mouse) ELISA Kit.

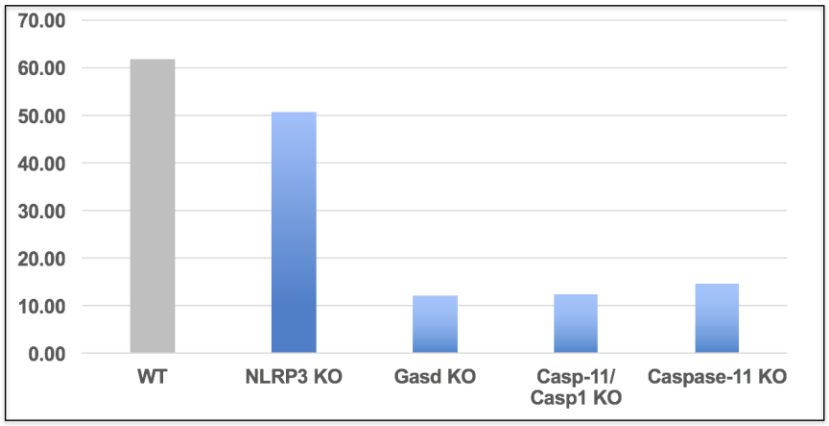

Figure 1: Detection of GSDMDC1 in supernatants of BMDMs transfected with LPS from different strains

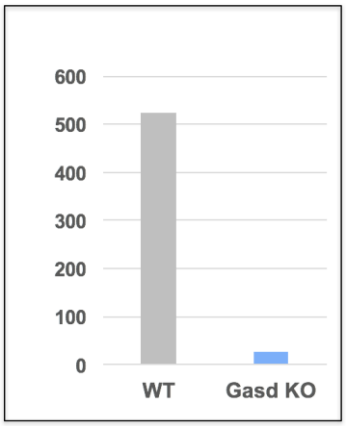

Figure 2: Detection of GSDMDC1 in cell extracts of BMDMs from WT and GSDMDC1 KO mice strains

D. Intra-assay precision:

Four samples of known concentrations of mouse GSDMDC1 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

E. Inter-assay precision:

Four samples of known concentrations of mouse GSDMDC1 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

12. Technical Hints and Limitations

- 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 100µl 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 TMB Solution protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

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. Notes