Human Gasdermin-A (GSDMA) ELISA Kit

Cat #: orb1670316 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Intended use: For the quantitative detection of Human Gasdermin- A (GSDMA) concentration in serum, plasma, urine and other biological fluids

Detectable Sample Type: serum, plasma, urine and other biological fluids

Sensitivity: 0.1 ng/ mL

Detection Range: 0.31-20 ng/mL

Background on GSDM

GSDM showed apoptotic activity upon expression in a human gastric cancer cell line. LMO1, RUNX3), and TGF- beta receptor II (TGFBR2) was coexpressed with GSDM in pit cells, and both LMO1 and RUNX3 were bound to the GSDM promoter in vitro. TGF- beta (TGFB1) upregulated LMO1 and GSDM expressions and induced apoptosis, and induction of apoptosis was inhibited by suppression of LMO1, RUNX3, and GSDM expressions. Saeki et al. (2007) concluded that GSDM is a component of TGF- beta signaling that induces apoptosis in gastric pit cells. The deduced 446 - amino acid protein contains a leucine zipper motif. Northern blot analysis detected Gsdm1 expression in mouse stomach, skin, and esophagus, but not in other tissues examined. Northern blot analysis of human tissues detected GSDM1 expression in stomach only.

Assay Principle

This assay employs a two- site sandwich ELISA to quantitate GSDMA in Human serum, plasma, urine. An antibody specific for GSDMA has been pre- coated onto a microplate. Standards and samples are pipetted into the wells and any GSDMA present is bound by the immobilized antibody. After removing any unbound substances, a biotin- conjugated antibody specific for GSDMA is added to the wells. After washing, Streptavidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin- enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GSDMA bound in the initial step. The color development is stopped and the intensity of the color is measured.

Kit Components

Reagents	Quantity	Reagents	Quantity
Assay plate (96 Wells)	1	Instruction manual	1
Standard (lyophilized)	2	Sample Diluent	1 x 20 mL
Biotin- Conjugate (concentrate 100 x)	1 x 120 µ L	Biotin- Conjugate Diluent	1 x 20 mL

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Streptavidin- HRP (concentrate 100 x)	1 x 120 µ L	Streptavidin- HRP Diluent	1 x 20 mL
Wash Buffer (concentrate 25 x)	1 x 20 mL	Substrate Solution	1 x 10 mL
Stop Solution	1 x 10 mL	Adhesive Films	4

Storage

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C if use within 2 months. Store at -20 °C if use after 2 months. Do not use expired kit.</td></tr>
<tr><td rowspan="10">Opened kit</td><td>Coated assay plate</td><td>Can be stored for 1 month at most at 2 - 8°C/ -20°C. Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal, and avoid the damp.</td></tr>
<tr><td>Standard</td><td rowspan="9">Can be stored for 1 at 2 - 8°C/ -20 °C. month at most</td></tr>
<tr><td>Biotin- Conjugate</td></tr>
<tr><td>Streptavidin- HRP</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Biotin- Conjugate Diluent</td></tr>
<tr><td>Streptavidin- HRP Diluent</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

***Once the standard is reconstituted it must be used immediately and cannot be stored for repeated use.**

Materials Required but Not Supplied

1. Microplate reader capable of measuring absorbance at 4 5 0 nm, with the correction wavelength set at 540 nm or 570 nm.
2. Precision single or multi- channel pipettes and disposable tips.
3. Deionized or distilled water.
4. Eppendorf Tubes for serial dilution samples.

5. Container for Wash Solution.

5. Absorbent paper for blotting the microtiter plate.

Sample Preparation

Sample Collection and Storage

Serum: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 2 - 8 ° C before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ - 2 0 ° C. Avoid repeated freeze- thaw cycles.

Plasma: Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ - 2 0 ° C. Avoid repeated freeze- thaw cycles.

Urine: Collect urine using a metabolic cage. Remove any particulates by centrifugation for 15 minutes at 1000 x g, 2- 8 °C and assay immediately or aliquot and store samples at - 2 0 ° C or - 8 0 ° C. Avoid repeated freeze- thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

Other biological fluids: Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at - 20 °C or -80 °C. Avoid repeated freeze/ thaw cycles.

Sample Dilution Proposal

Human serum, plasma or urine samples require no dilution before test. **The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.**

Notes

1. Samples to be used within 5 days may be stored at 2-8 °C, otherwise samples must be stored at - 20 °C (≤ 1 month) or - 80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.

2. Sample hemolysis will influence the result, so hemolytic specimens cannot be detected.

3. When performing the assay, bring samples to room temperature.

Reagent Preparation

Bring all reagents to room temperature before use.

Wash Buffer (1 x) - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 2 0 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).

Biotin- Conjugate (1 x) - Centrifuge the vial before opening.

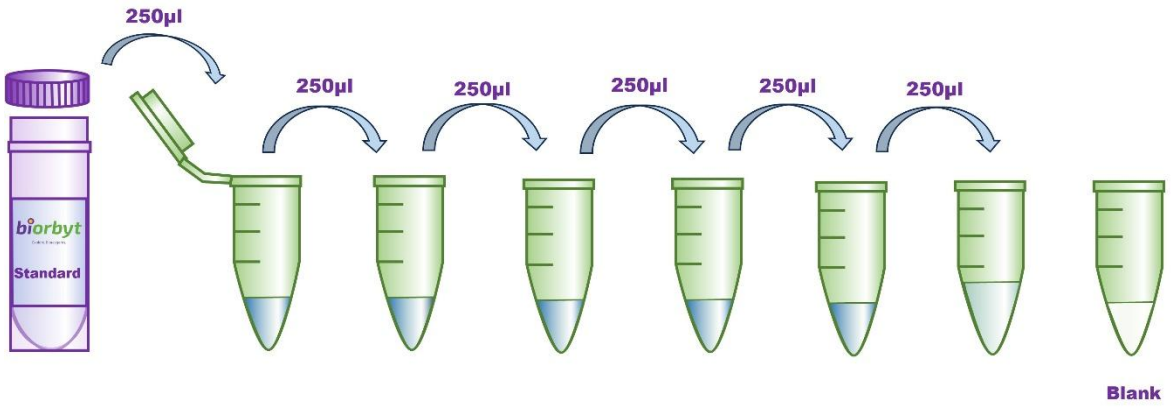
Biotin- Conjugate requires a 10 0 - fold dilution. A suggested 10 0 - fold dilution is 10 µ L of Biotin-Conjugate (100 x) + 990 µ L of Biotin-Conjugate Diluent

Streptavidin- HRP (1 x) - Centrifuge the vial before opening.

Streptavidin- HRP requires a 100 - fold dilution. A suggested 100 - fold dilution is 10 µ L of Streptavidin-HRP (100 x) + 990 µL of Streptavidin-HRP Diluent.

GSDMA Standard - Centrifuge the standard vial at 6000 - 10000 rpm for 30 s. Reconstitute the Standard with 1 mL of Sample Diluent. Swirl or mix gently to ensure complete homogeneous solubilization (concentration of reconstituted standard = 20 ng/mL). The standard has to be used immediately after reconstitution and cannot be stored.

Use Eppendorf Tubes - Pipette 2 5 0 µ L of the Sample Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (20 ng/ mL). The Sample Diluent serves as the zero standard (0 ng/ mL).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ mL	20	10	5	2.5	1.25	0.625	0.31	0

Assay Procedure

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2 - 8 °C.

3. Add 100 µ L of standard and sample per well. Cover with the adhesive films provided. Incubate for 2 hours at 37 ° C. A plate layout is provided to record standards and samples assayed.

4. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 µ L) using a squirt bottle, multi- channel pipette, manifold dispenser or autowasher. 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.

5. Add 100 µ L of Biotin-Conjugate (1 x) to each well. Cover with the adhesive films. Incubate for

1 hour at 37 °C. (Biotin- Conjugate (1 x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)

6. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 µ L) using a squirt bottle, multi- channel pipette, manifold dispenser or autowasher. 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.

7. Add 100 µ L of Streptavidin- HRP (1 x) to each well. Cover the microtiter plate with the adhesive films. Incubate for 1 hour at 37 °C.

8. Aspirate each well and wash, repeating the process for a total of five washes. Wash by filling each well with Wash Buffer (250 µ L) using a squirt bottle, multi- channel pipette, manifold dispenser or autowasher. 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.

9. Add 100 µ L of Substrate Solution to each well. Incubate for 15-20 minutes at 37 °C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.

10. Add 50 µL of Stop Solution to each well. When the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. 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 and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data 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 x- axis against the concentration on the y- axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the GSDMA 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.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Performance

Detection range

0.31 ng/ mL - 20 ng/ mL. The standard curve concentrations used for the ELISA' s were 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.31 ng/mL, 0 ng/mL.

Sensitivity

The limit of detection of Human GSDMA defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.1 ng/ mL (mean of 6 independent assays)

Specificity

This assay has high sensitivity and excellent specificity for detection of Human GSDMA. No significant cross-reactivity or interference between Human GSDMA and analogues were observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between Human GSDMA and all the analogues, therefore, cross reaction may still exist.

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)

Three samples of known concentration were tested in forty separate assays to assess inter- assay precision.

CV (%) = SD/meanX100

Intra- Assay: CV<8 %

Inter- Assay: CV<12%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage conditions.

The loss rate was determined by accelerated thermal degradation test. Keep the kit at 37 °C for 4

and 7 days and compare O.D. values of the kit kept at 37 °C with that of at recommended temperature.

Note:

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

ELISA Troubleshooting

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.

linear but the background is too high	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Color development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample enzyme contaminated addition, the marker is and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.) and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3

	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.
	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.

	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The color of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

Declaration

1. The instruction manual also suits the kit of 48 T, but all reagents of 48T kit are reduced by half.
2. There may be some foggy substances in the wells when the plate is open for the first time. It will not effect on the final assay results. Do not remove microtiter plates from the storage bag unless needed.
3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

4. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as far as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 1 0 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

5. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

6. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/ or rotating the plate 180 degrees between wash steps may improve assay precision.

7. Controlling of reaction time: Observe the change of color after adding Substrate Solution (e. g. observation once every 1 0 minutes), Substrate Solution should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

8. Substrate Solution is easily contaminated. Substrate Solution should remain colorless or light blue until added to the plate. Please protect it from light.

9. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

10. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganisms.

11. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10 nm or less and an optical density range of 0- 3 O. D. or greater at 450 ± 10 nm wavelength is acceptable for use in absorbance measurement.

12. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.

13. Limited by the current condition and scientific technology, we can' t completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So, there might be some qualitative and technical risks to using the kit.

14. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.

15. Kits from different batches may be a little different in detection range, sensitivity and color developing time.

16. Each kit has been strictly passed QC test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment's. Intra-assay variance among kits from different batches might arise from above factors, too.

17. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.

18. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

19. Valid period: six months.

Limitations of The Procedure

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. The kit should not be used beyond the expiration date.
3. Do not mix or substitute reagents with those from other lots or sources.
4. It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
6. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
7. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the ELISA Kit, the possibility of interference cannot be excluded.

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