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USER MANUAL



ELISA KIT

Human GNLY(Granulysin) ELISA Kit

Catalog No.tcee1380 96 Tests

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

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Human GNLY(Granulysin) ELISA Kit
Catalog No.tcee1380

Available Sizes

Size: 96 Tests

Specifications

Research Area:	Immunology
Species Reactivity:	Human
Sample Type:	Serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids
Sensitivity:	0.14ng/mL
Recovery:	Recovery ranges are from 89-99% .
Detection Range:	0.23-15ng/mL
Assay Time:	4.5 hrs
Detection Method:	Sandwich ELISA

INTENDED USE

It's used for in vitro quantitative determination of Human GNLY concentrations in serum, plasma and other biological fluids.

TEST PRINCIPLE

The method to test Human GNLY is typical Sandwich-ELISA. The plate was first pre-coated with Human GNLY antibody. When standards or samples are combined with the coated antibody, the detection antibody which labeled with biotin combines to standard or samples next, then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively. The substrate solution TMB is used for color developing to appear blue.
Finally the color changes to yellow by the acid(stop solution) function.
The concentration of Human GNLY is in direct proportion to the color shade which calculated by optical density.

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Materials and Storage.

1. The unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, take out the Micro ELISA Plate, Reference Standard, Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate to store at -20℃, the rest components are still stored at 4℃
2. **For used kit:** When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.

Components

Item	Size	Notice
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	4℃ for 1 month, -20℃ for 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	120 uL	
Concentrated HRP Conjugate (100×)	120 µL	4℃ for 1 month, -20℃ for 6 months (shading light)
Reference Standard & Sample Diluent	20 mL	4℃ for 6 months
Biotinylated Detection Ab Diluent	14 mL	
HRP Conjugate Diluent	14 mL	
Concentrated Wash Buffer (25×)	30 mL	
Substrate Reagent	10 mL	4℃ for 6 months (shading light)
Stop Solution	10 mL	
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Materials and instruments required but not supplied

- Microplate reader with 450 ± 10nm filter.
- Single or multi-channel pipettes with high precision and disposable tips.
- Microcentrifuge Tubes.
- Deionized or distilled water.
- Absorbent paper for blotting the microplate.
- Container for Wash Solution.
- 37℃ Incubator.

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Sample Collection and Storage

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 ℃ before centrifugation for 20 minutes at approximately 1,000×g.
Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant.
Centrifuge samples for 15 minutes at 1,000×g at 2-8℃ within 30 minutes of collection.
Remove plasma and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (catalog: tcis1988, different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifugated for 5 minutes at 10,000×g.

Remove the supernatant and assay immediately or aliquot and store at ≤-20℃

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8oC to remove cellular debris. Assay immediately or aliquot and store at ≤-20℃

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernant and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4 ℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃ (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

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Reagent Preparation and Storage

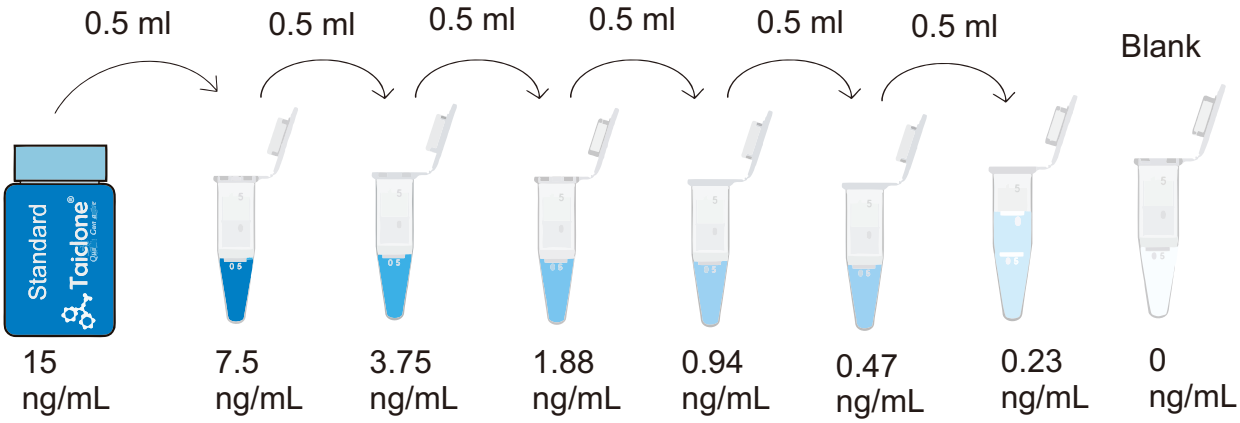
Bring all reagents to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

1. Wash Solution:

Dilute 20mL of Concentrated Wash Solution (30X) into 580 mL with deionized or distilled water to prepare 600mL of wash Solution (1x)
Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2. Standard:

Reconstitute the Standard with 1.0 mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 15ng/ml. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 15、7.5、3.75、1.88、0.94、0.47、0.23、0 ng/mL.
If you want to make standard solution at the concentration of 7.5ng/mL working solution, you should take 500 µL of standard at 15ng/mL, add it to an EP tube with 500 µL of Reference Standard &Sample Diluent, and mix it. Procedures to prepare the remained concentrations are all the same.
The Reference Standard &Sample Diluent serves as the zero (0 ng/mL)..



3. Biotinylated Detection Ab working solution: -

Centrifuge the stock solution before use, dilute the stock solution to working solution with Biotinylated Detection Ab Diluent (1:99).

4. Concentrated HRP Conjugate working solution:

- Dilute the Concentrated HRP Conjugate to working solution with Concentrated HRP Conjugate Diluent (1:99)

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5. Wash Solution-

: Add 720 mL of deionized or distilled water to 30 mL of Concentrated Wash Buffer to prepare the wash working solution.

Plate Wash Method

:

1. **Wash manually:** Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper.
2. **Wash with a microplate washer:** Set parameters (liquid volume: 350uL; soaking time: 1~2 min; wash time: 3 or 5 times), put the plate to washer and start.

Assay Procedure:

1. **Add Sample:** Add 100µL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer as provided. Incubate for 90 minutes at 37℃.
2. **Biotinylated Detection Ab**: Remove the liquid of each well, don't wash. Immediately add 100µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C
3. **Wash:** Wash for 3 times.
4. **HRP Conjugate:** Add 100µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.
5. **Wash**: Wash for 5 times.
6. **Substrate:** Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, user should terminate the reaction.
7. **Stop:** Add 50µLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.
8. **OD Measurement:** Determine the optical density (OD value) of each well at once, using a microplate reader set to 450 nm

NOTE For Assay Procedure:

1. **ELISA Plate:** The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
2. **Add Sample:** The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended
3. **Incubation:** To prevent evaporation and 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. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.
4. **Washing:** The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader

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5. **Reagent Preparation:** As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions.
   To minimize imprecision caused by pipetting, ensure that pipettors are calibrated.
   It is recommended to suck more than 10 μL for once pipetting.
   Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into a small pack according to the amount of each assay, keep them at -20 -80 and avoid repeated freezing and thawing.
6. **Reaction Time Control**: Please control reaction time strictly following this product description!
7. **Substrate**: Substrate Solution is easily contaminated. Please protect it from light.
8. Stop Solution: As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.
9. **Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.
10. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
11. **Do not use components from different batches of kit** (washing buffer and stop solution can be an exception).
12. **To avoid cross-contamination**, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

Calculation of result.

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve on log-log graph paper, with the concentration on the x-axis and the OD values on the y-axis. Using some software such as OriginPro 8.0 can be also used to calculate the result.
If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

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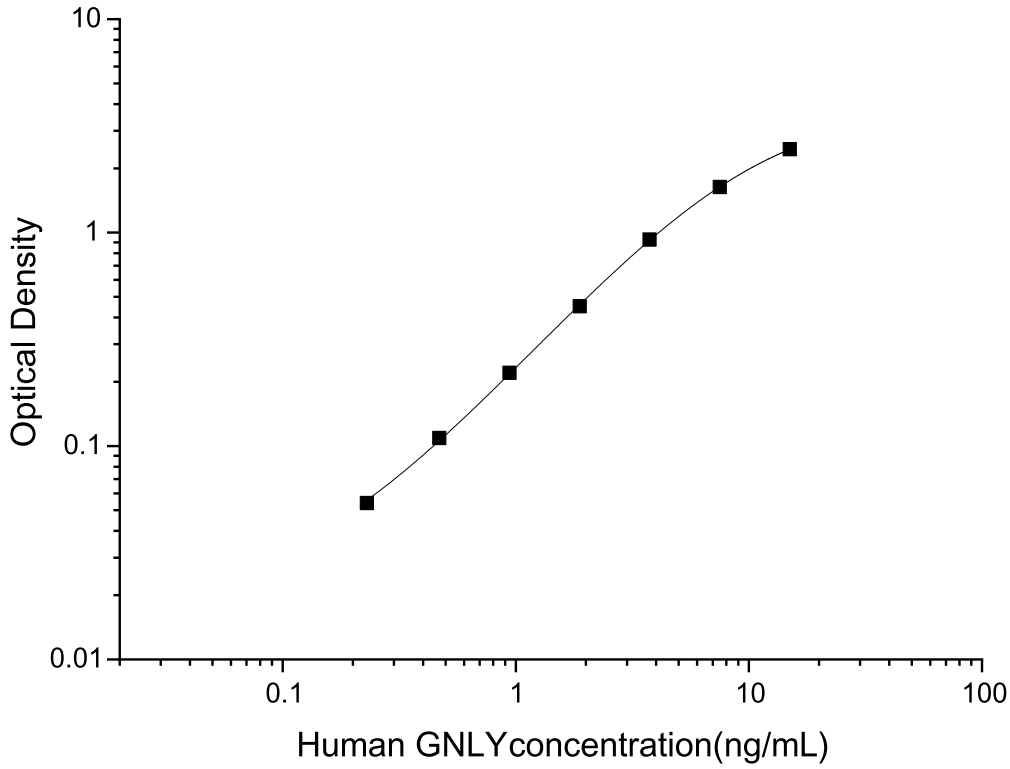
Typical Data & Standard Curve

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), plotting log of the data to establish standard curve for each test is recommended. Typical standard curve below is provided for reference only.

Concentration(ng/mL)	15	7.5	3.75	1.88	0.94	0.47	0.23	0
OD	2.513	1.69	0.982	0.506	0.275	0.164	0.109	0.055
Corrected OD	2.458	1.635	0.927	0.451	0.22	0.109	0.054	-



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Recovery

The recovery of Human GNLY spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	94-106	99
EDTA plasma (n=5)	90-105	97
Cell culture media (n=5)	89-100	94

Linearity

Samples were spiked with high concentrations of Human GNLY and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)
1:2	Range (%)	88-104	93-107
	Average (%)	95	101
1:4	Range (%)	89-103	85-96
	Average (%)	97	90
1:8	Range (%)	93-107	88-99
	Average (%)	99	93
1:16	Range (%)	90-102	84-98
	Average (%)	97	90

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human GNLY were tested 20 times on one plate, respectively.
Mean Intra-CV: 5.56%
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human GNLY were tested on 3 different plates, 20 replicates in each plate.
Mean Inter-CV: 4.48%

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Important Note

1. Limited by the current conditions 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 use the kit.
2. The final experimental results will be closely related to validity of the products, so the kit should be used prior to the expiration date. And please store the kits exactly according to the instruction.
3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website is only for reference.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. 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 microorganism. TMB Substrate should remain colorless till it is reacted with the enzyme which binds to the microplate.
6. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microplate from the storage bag until needed.
7. 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 reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
8. Variation in sample preparation and each step of experimental operation may cause different results. In order to get better reproducible results, the operation of each step in the assay should be controlled.
9. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.
11. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
12. Please predict the concentration of target molecules in samples, or arrange a preliminary experiment, it is a good way to solve specific problem, e.g. the concentration of samples are beyond the detection range of the kit.
13. The kit might not be suitable for detection of samples from some special experiment, for instance, knock-out experiments, due to their uncertainty of effectiveness.
14. The instruction manual is also for the kit of 48T, but all reagents of 48T kit are reduced by half.
15. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.

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PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution.
Wear eye, hand, face, and clothing protection when using this materials.

Trouble Shooting

Problem	Possible Source	Correction Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and Add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate &substrate,color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Use new sample and repeat assay

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