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γ-Glutamyl Transferase Activity Assay Kit

Item No. 702210

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400514	GGT Assay Buffer	1 vial/25 ml	-20°C
400515	*p*NA Standard	1 vial/50 µl	-20°C
400516	GGT Substrate	1 vial	-20°C
400517	GGT Substrate Diluent	1 vial/3.5 ml	-20°C
400518	GGT Positive Control	1 vial	-20°C
700020	Half-Volume 96-Well Plate	1 plate	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 418 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended

INTRODUCTION

Background

γ-Glutamyl transferase (GGT) is a glycosylated enzyme that transfers γ-glutamyl moieties from proteins and other targets, such as glutathione, to an acceptor, such as an amino acid.[1] It is found in a variety of tissues, including the liver, kidneys, and gallbladder, and is localized to the plasma membrane.[1,2] GGT has a role in glutathione recycling, where it removes γ-glutamyl moieties from glutathione and glutathione conjugates to allow their transport into cells to form glutathione and mercapturic acids.[1,3]

Serum levels of GGT are increased in patients with chronic active hepatitis, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease.[1,2] GGT levels have been used as markers of liver disease, but they can also be elevated in non-hepatic diseases, such as cholestasis, pancreatitis, and diabetes, and illnesses, such as COVID-19.[1,4] GGT serum levels are reduced in patients with low GGT familial intrahepatic cholestasis, a genetic disorder characterized by mutations in the gene encoding the bile salt export pump (BSEP).[1,5]

About This Assay

Cayman's GGT Activity Assay Kit provides a convenient method of detecting GGT activity in plasma, serum, and tissue lysates. In this assay, GGT catalyzes the transfer of the γ-glutamyl group from L-γ-glutamyl-*p*-nitroaniline (γG*p*NA) to glycylglycine resulting in the liberation of the colored product *p*-nitroaniline (*p*NA), which can be measured at 418 nm. Under circumstances in which the GGT activity rate is limiting, the rate of increase is directly proportional to the GGT activity in the sample.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **GGT Assay Buffer - (Item No. 400514)**

   This vial contains 25 ml of GGT Assay Buffer. Warm the buffer to room temperature before use. The reagent is ready to use as supplied and, once thawed, will be stable for one week when stored at 4°C. For longer-term storage, freeze at -20°C and use within three months.

2. **GGT Substrate Diluent - (Item No. 400517)**

   This vial contains 3.5 ml of GGT Substrate Diluent. Warm the GGT Substrate Diluent to room temperature prior to use.

3. **GGT Substrate - (Item No. 400516)**

   This vial contains a crystalline GGT substrate. Reconstitute the GGT Substrate with 3 ml of the room temperature GGT Substrate Diluent immediately prior to use. Vortex briefly and confirm the reagent has dissolved prior to preparing the GGT Reaction Buffer. Reconstituted GGT Substrate is unstable at room temperature and should be used within one hour of reconstitution. Any unused reconstituted substrate should be stored immediately at -20°C and used within one month.

4. ***p*NA Standard - (Item No. 400515)**

   This vial contains 50 µl of 50 mM *p*NA in DMSO. To prepare a working standard solution, warm the vial to 37°C until crystals are dissolved completely. In a fresh tube, dilute 20 µl of *p*NA Standard with 980 µl of GGT Assay Buffer to generate a 1 mM working standard solution. The working standard solution must be used the same day. If all of the undiluted *p*NA will not be used at one time, aliquot the undiluted *p*NA Standard and store at -20°C where it will be stable for at least two months.

5. **GGT Positive Control - (Item No. 400518)**

   This vial contains lyophilized GGT from bovine kidney. Reconstitute the contents of the vial with 0.25 ml of GGT Assay Buffer by pipetting gently up and down several times. Store the reconstituted GGT Positive Control on ice prior to use. Any unused reconstituted protein may be stored at -20°C for up to one month.

6. **GGT Reaction Buffer**

   If assaying a full plate, dilute 2.8 ml of reconstituted GGT Substrate with 9.8 ml of GGT Assay Buffer to generate the GGT Reaction Buffer. If assaying a partial plate, calculate the volume of GGT Reaction Buffer required (90 µl/well) then prepare the GGT Reaction Buffer by diluting 2 parts reconstituted GGT Substrate with 7 parts GGT Assay Buffer. Store the prepared GGT Reaction Buffer at room temperature and use in the assay within one hour.

Sample Preparation

Plasma

1. Collect blood using an anticoagulant, such as heparin or citrate. EDTA is unsuitable as an anticoagulant for this assay.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, store at -80°C. Avoid repeated freeze/thaw cycles.
3. Plasma does not need to be diluted prior to use in the assay.

Serum

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, store at -80°C. Avoid repeated freeze/thaw cycles.
4. Serum does not need to be diluted prior to use in the assay.

Tissue Homogenate

1. Prior to dissection, rinse the tissue with PBS, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 5 ml of cold GGT Assay Buffer per 250 mg of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -20°C.

NOTE: If the rate of increase in absorbance at a wavelength of 418 nm (A418) is greater than 0.05 absorbance units/min, dilution of the sample with GGT Assay Buffer will be necessary to fall within the linear range of the assay.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is recommended that at least one well be designated for the GGT Positive Control and one well for the Reaction Background. It is suggested that each sample, the GGT Positive Control, and Reaction Background be assayed in duplicate and that the contents of each well are recorded on the template sheet provided on page 21. A typical layout of samples to be measured in duplicate is provided below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	1	1	9	9	17	17	25	25	33	33
B	RB	RB	2	2	10	10	18	18	26	26	34	34
C	S1	S1	3	3	11	11	19	19	27	27	35	35
D	S2	S2	4	4	12	12	20	20	28	28	36	36
E	S3	S3	5	5	13	13	21	21	29	29	37	37
F	S4	S4	6	6	14	14	22	22	30	30	38	38
G	S5	S5	7	7	15	15	23	23	31	31	39	39
H	S6	S6	8	8	16	16	24	24	32	32	40	40

PC - Positive Control Wells
RB - Reaction Background Wells
S1-S6 - Standard Wells
1-40 - Sample Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 100 µl in all the wells.
- All reagents must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in duplicate, but it is at the user's discretion to do so.
- 26 samples can be assayed in triplicate or 40 in duplicate.
- The assay is performed at 37°C.
- Monitor the absorbance at 418 nm.

Performing the Assay

1. **Standard Wells**

   Prepare the standards in the standard wells of the Half-Volume 96-Well Plate (Item No. 700020; see **Sample plate format**, Figure 1, page 9) using the 1 mM working standard solution and GGT Assay Buffer according to the table below.

*p*NA per well (nmol)	1 mM Working Standard Solution (µl)	GGT Assay Buffer (µl)
50	50	50
40	40	60
30	30	70
20	20	80
10	10	90
0	0	100

Table 1. Preparation of the *p*NA standards

2. **Positive Control Wells**

   Add 10 µl of GGT Positive Control to the designated wells on the plate.

3. **Sample Wells**

   Add 10 µl of sample to the designated wells on the plate.

4. **Reaction Background Wells**

   Add 10 µl of GGT Assay Buffer to the designated wells on the plate.

Well	GGT Assay Buffer	Positive Control	Sample	GGT Reaction Buffer
Positive Control	--	10 µl	--	90 µl
Sample	--	--	10 µl	90 µl
Reaction Background	10 µl	--	--	90 µl

Table 2. Pipetting summary

5. Initiate the reactions by adding 90 µl of GGT Reaction Buffer to all of the wells being used. Do not add GGT Reaction Buffer to the standard wells.
6. Immediately measure the absorbance at 418 nm once every minute at 37°C. Incubation times will depend on the activity of the samples. It is recommended to monitor the assay using a kinetic method to identify the portion of the reaction within the linear range of the standard curve. Alternatively, the reaction can be monitored in endpoint mode by collecting an initial absorbance (A_0) reading after 10 minutes, followed by a second measurement after 40 minutes (A_1). For low-activity samples, users can continue reading the assay up to 120 minutes.

ANALYSIS

Calculations

1. Plot the *p*NA standard curve then determine the slope. Plot the final absorbance values for the standard wells on the y-axis and the amount of *p*NA (nmoles) per well on the x-axis.
2. Determine the change in absorbance (ΔA_{418}) per minute in the reaction wells and background wells:

   Plot the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve. Subtract the background slope from the reaction slope to obtain the background-corrected slope.

   OR

   Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

$$\frac{(A_1 - \text{reaction background } A_1) - (A_0 - \text{reaction background } A_0)}{\text{time (min)}} = \Delta A/\text{min}$$

3. Use the following formula to calculate the GGT activity:

$$(\Delta A/\text{min}/p\text{NA slope}) \times 100 = \text{nmol/min/ml} = \text{mU/ml}$$

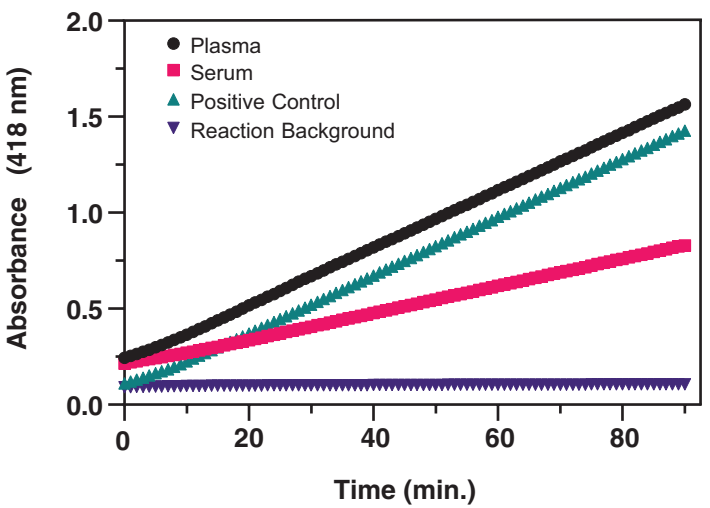


Figure 2. GGT activity in plasma and serum and for the bovine kidney GGT Positive Control

Performance Characteristics

Spike and Recovery

Human samples with low GGT activity ("Low") were spiked with samples with increased GGT activity ("High") and analyzed using the GGT Activity Assay Kit. The results are shown below.

% "High" Plasma	% "Low" Plasma	Measured Activity (nmol/min/well)	Expected Activity (nmol/min/well)	% Recovery
Plasma (heparin)				
0	100	0.114	0.114	--
20	80	0.177	0.179	99%
40	60	0.241	0.244	99%
60	40	0.309	0.309	100%
80	20	0.378	0.374	101%
100	0	0.438	0.438	--
Serum				
0	100	0.107	0.107	--
20	80	0.177	0.169	99%
40	60	0.233	0.231	99%
60	40	0.300	0.293	100%
80	20	0.351	0.355	101%
100	0	0.417	0.417	--

Table 3. Spike and recovery of GGT in plasma and serum

Linearity

Plasma, serum, and kidney tissue extract were processed as described in the **Sample Preparation** section (see page 7), then serially diluted with GGT Assay Buffer and evaluated for linearity with the GGT Activity Assay Kit. The results are shown in Table 4 below and continued on page 17.

Dilution	Measured GGT Activity (nmol/min./well) Dilution Adjusted	Linearity (%)
Plasma (heparin)		
1	0.383	100
2	0.384	100
4	0.378	98
8	0.377	90
16	0.339	88
32	0.394	102
Serum		
1	0.417	100
2	0.406	97
4	0.383	92
8	0.409	98
Tissue Extract		
1	2.306	100
2	2.531	109
4	2.617	113
8	2.727	118
16	2.716	118
32	2.643	115

Table 4. Linearity in various matrices

*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

Sensitivity

The lower limit of quantification (LLOQ) for this assay is 0.02 nmol/min/ml (mU/ml).

Precision:

When a series of 16 GGT activity measurements were performed in plasma and serum on the same day under the same experimental conditions, the intra-assay coefficients of variation were 4 and 3%, respectively. When a series of sample measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 7% in both plasma and serum.

Interferences

The following reagents were tested for interference in the assay.

Reagent		Will Interfere (Yes or No)
Detergents	SDS (0.1%)	Yes
	Triton X-100 (≤0.1%)	No
	Tween 20 (≤0.1%)	No
	NP40 (≤0.1%)	No
Chelators/protease inhibitors	EDTA (1 mM)	Yes
	EGTA (1 mM)	Yes
	AEBSF (≤0.1% mM)	No
	Bestatin (≤5 µM)	No
	Leupeptin (≤2 µM)	No
	Pepstatin A (≤1 µM)	No
Other	BSA (≤0.25%)	No
	DMSO (1%)	Yes
	Ethanol (≤1%)	No

Table 5. Interferences

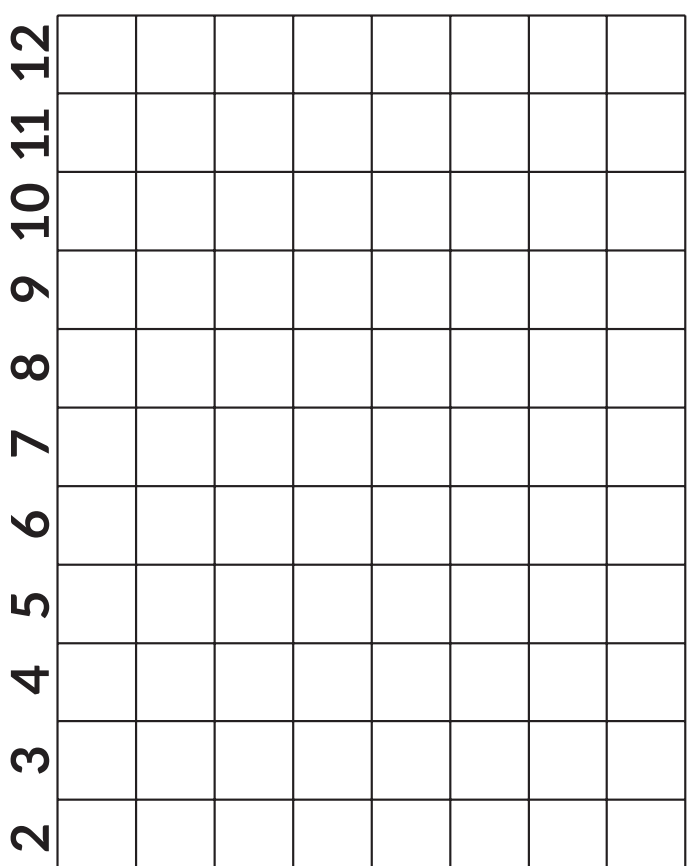
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No increase in absorbance	A. Sample was not added to the wells B. GGT activity is too low to detect	A. Make sure to add all the components to the well(s) and re-assay B. Concentrate the sample with an Amicon concentrator with a MW cut-off of 10 kDa and re-assay
High reaction background absorbance (>0.5)	Reconstituted substrate has been stored inappropriately.	Reconstituted substrate should be used within one hour or stored at -20°C for up to one month.

References

1. Brennan, P.N., Dillon, J.F., and Tapper, E.B. Gamma-glutamyl transferase (γ-GT) - an old dog with new tricks? *Liver Int.* **42(1),** 9-15 (2021).
2. Kunutsor, S.K. Gamma-glutamyltransferase-friend or foe within? *Liver Int.* **36(12),** 1723-1734 (2016).
3. Bachhawat, A.K. and Yadav, S. The glutathione cycle: Glutathione metabolism beyond the γ-glutamyl cycle. *IUBMB Life* **70(7),** 585-592 (2018).
4. Kumar-M, P., Mishra, S., Jha, D.K., *et al.* Coronavirus disease (COVID-19) and the liver: a comprehensive systematic review and meta-analysis. *Hepatol. Int.* **14(5),** 711-722 (2020).
5. van Wessel, D.B.E., Thompson, R.J., Gonzales, E., *et al.* Genotype correlates with the natural history of severe bile salt export pump deficiency. *J. Hepatol.* **73(1),** 84-93 (2020).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.