

L-Theanine Fluorometric Assay Kit

Item No. 700570

TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Precautions
	4	If You Have Problems
	4	Storage and Stability
	4	Materials Needed but Not Supplied
INTRODUCTION	5	Background
	5	About This Assay
PRE-ASSAY PREPARATION	6	Reagent Preparation
	7	Sample Preparation
ASSAY PROTOCOL	9	Plate Set Up
	11	Standard Preparation
	12	Performing the Assay
ANALYSIS	13	Calculations
	13	Performance Characteristics
RESOURCES	15	Troubleshooting
	16	References
	17	Related Products
	18	Warranty and Limitation of Remedy
	19	Plate Template
	20	Notes

GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size
700571	L-Theanine Standard	2 vials/250 µl
700572	L-Theanine Assay Buffer (5X)	1 vial/6 ml
700573	L-Theanine Initiator	2 vials
700574	L-Theanine Detector	2 vials/100 µl
700575	Methanol Assay Reagent	1 vial/8 ml
400017	96-Well Solid Plate (black)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

It is recommended to take appropriate precautions when using the kit reagents (i.e., lab coat, gloves, eye goggles, etc.) as some of them can be harmful.

Sodium cyanide is toxic if inhaled, ingested, or if in contact with skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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 at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 430-440 nm and an emission wavelength of 485-495 nm
2. Adjustable pipettors and a repeat pipettor
3. A source of pure water. Glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

L-Theanine (γ -glutamylethylamide, γ -ethylamino-L-glutamic acid, glutamic acid γ -ethyl amide, or 5-N-ethyl-glutamine) is the major amino acid found in *Camellia sinensis*, the source of green tea. It can also be found in other *Camellia* species (*C. japonica* and *C. sasanqua*) and in the mushroom *Xerocomus badius*. L-Theanine is water-soluble and exists only in the free (non-protein) form. It comprises as much as 50% of the plant's total free amino acids and 1-2% of the dry weight of the leaves.¹ L-Theanine is the component in green tea which gives it the exotic taste known as umami.² L-Theanine does not occur naturally in humans.

L-Theanine has been shown to have positive effects on the immune system responsiveness to infection by boosting the $\gamma\delta$ T-cells infection-fighting abilities to recognize the antigen ethylamine, which is a renal metabolite of L-Theanine.³ In human subjects, L-Theanine has been shown to increase the production of brain α -waves, which causes a state of relaxed alertness.^{1,3} In a placebo-controlled clinical trial, the addition of L-Theanine to antipsychotic medication was helpful in reducing some symptoms of schizophrenia.⁴ L-Theanine also offers a neuroprotective effect by binding to glutamate receptors and their receptor subtypes (i.e., N-methyl-D-aspartate [NMDA]), preventing excess calcium release into the intercellular spaces.³ In animal models, L-Theanine may be effective as a biochemical modulator for select anthracyclines.³ In spontaneously hypertensive rats, L-Theanine has demonstrated antihypertensive properties by significantly decreasing blood pressure.³ In rat studies, L-Theanine increases the levels of dopamine and decreases the levels of norepinephrine and serotonin in the brain.³

About This Assay

Cayman's L-Theanine Fluorometric Assay provides a simple, reproducible, and sensitive tool for assaying L-Theanine from plasma, serum, and tea. L-Theanine forms a fluorescent complex with the detector in the presence of cyanide, which can be analyzed at an excitation wavelength of 430-440 nm and an emission wavelength of 485-495 nm.

Reagent Preparation

1. L-Theanine Standard - (Item No. 700571)

Each vial contains 250 μ l of a 1 mM L-Theanine solution.

2. L-Theanine Assay Buffer (5X) - (Item No. 700572)

The vial contains 6 ml of 50 mM sodium borate, pH 9.2. Thaw the vial at room temperature and bring the volume to 30 ml with HPLC-grade water. When stored at 4°C, the diluted Assay Buffer (10 mM sodium borate, pH 9.2) is stable for at least six months.

3. L-Theanine Initiator - (Item No. 700573)

Each vial contains 1.5 mg of sodium cyanide; see **Precautions** section before handling. Reconstitute the contents of the vial with 3 ml of the diluted Assay Buffer. One vial is enough reagent to assay 60 wells. If additional wells are being utilized, then reconstitute the second vial. The reconstituted solution is stable for one hour.

4. L-Theanine Detector - (Item No. 700574)

Each vial contains 100 μ l of L-Theanine detector. Prior to use, thaw one vial at room temperature. Add the contents of the vial to a test tube containing 2.9 ml of methanol. One vial is enough detector to assay 60 wells. If additional wells are being utilized, then thaw the second vial and dilute with methanol. The diluted detector solution is stable for one hour.

5. Methanol Assay Reagent - (Item No. 700575)

The vial contains 8 ml of a methanol. It is ready to use in the assay.

Sample Preparation

In order to determine L-Theanine in plasma or serum, a 'baseline' sample should be taken prior to ingestion of L-Theanine. Baseline samples cannot be taken if L-Theanine is suspected to be in the system. Samples can be collected after 48-72 hours free from L-Theanine ingestion. Retain a sample of tea to quantify the amount of L-Theanine ingested.

After ingestion of L-Theanine, plasma or serum samples should be collected at pre-determined time periods (it is suggested that sample collection times should begin at 45-60 minutes, but that could depend upon the model being used). The timed samples are then compared to the 'baseline' plasma or serum sample collected prior to ingestion of the L-Theanine. An example of a time study is shown in Figure 1.

L-Theanine cannot be found in urine samples, since it is thought to be hydrolyzed to glutamic acid (L-glutamate) and ethylamine in the kidneys by phosphate-independent glutaminase.³

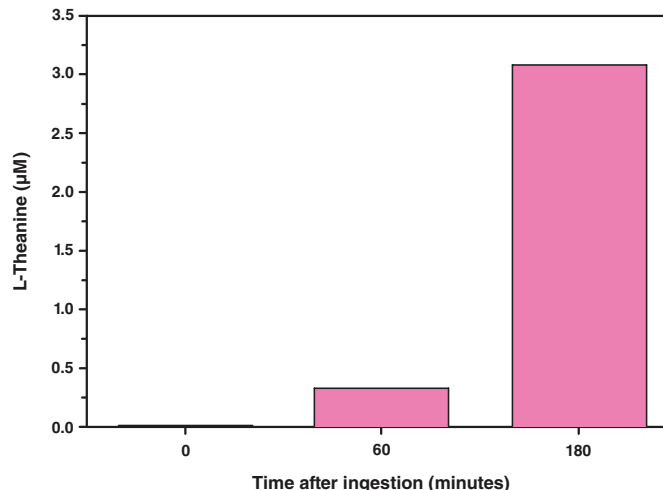


Figure 1. Time evaluation. One green tea bag was seeped in 277 ml of hot water for one hour. A plasma sample was drawn before ingesting the entire 277 ml of tea containing 11.9 mg of L-Theanine. Plasma was drawn at 60 and 180 minutes after ingestion. L-Theanine concentration was determined in the pre-ingestion plasma and in the 60 and 180 minute plasma samples.

Plasma

1. Collect blood using an anticoagulant such as heparin, EDTA or sodium citrate.
2. Centrifuge the blood at 700-1000 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, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Typically, plasma samples require a dilution of at least 1:100 or greater. Dilute the plasma samples using the diluted Assay Buffer before assaying.

Serum

1. Collect blood without using an anticoagulant.
2. Allow the 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, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Typically, serum samples require a dilution of at least 1:100 or greater. Dilute the serum samples using the diluted Assay Buffer before assaying.

Tea

1. Place one tea bag (or weighed amount of tea) into a beaker.
2. Add 12 ounces (355 ml) of boiling water.
3. Allow the tea to steep for desired time point.
4. After the desired time point has been reached, remove tea bag and stir tea until homogeneous.
5. Aliquot the tea into micro-centrifuge tubes. Store tea on ice. If not assaying the same day, freeze at -80°C. The tea sample will be stable for one month while stored at -80°C.
6. Typically tea samples require dilutions of at least 1:10 or greater. Dilute the tea samples using the diluted Assay Buffer before assaying.

Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that each sample and standard be assayed at least in duplicate (triplicate is preferred). A typical layout of samples to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 19).

	1	2	3	4	5	6	7	8	9	10	11	12
A	(A)	(A)	(S1)	(S1)	(S9)	(S9)	(S17)	(S17)	(S25)	(S25)	(S33)	(S33)
B	(B)	(B)	(S2)	(S2)	(S10)	(S10)	(S18)	(S18)	(S26)	(S26)	(S34)	(S34)
C	(C)	(C)	(S3)	(S3)	(S11)	(S11)	(S19)	(S19)	(S27)	(S27)	(S35)	(S35)
D	(D)	(D)	(S4)	(S4)	(S12)	(S12)	(S20)	(S20)	(S28)	(S28)	(S36)	(S36)
E	(E)	(E)	(S5)	(S5)	(S13)	(S13)	(S21)	(S21)	(S29)	(S29)	(S37)	(S37)
F	(F)	(F)	(S6)	(S6)	(S14)	(S14)	(S22)	(S22)	(S30)	(S30)	(S38)	(S38)
G	(G)	(G)	(S7)	(S7)	(S15)	(S15)	(S23)	(S23)	(S31)	(S31)	(S39)	(S39)
H	(H)	(H)	(S8)	(S8)	(S16)	(S16)	(S24)	(S24)	(S32)	(S32)	(S40)	(S40)

A-H = Standards
S1-S40 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor 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 150 μ l in all wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate (triplicate is preferred), but it is the user's discretion to do so.
- The assay is performed at 25°C.
- Monitor the fluorescence with an excitation wavelength of 430-440 nm and an emission wavelength of 485-495 nm.

Standard Preparation

Take eight clean test tubes or polystyrene tubes and label them A-H. Add the amount of 1 mM L-Theanine Standard and diluted Assay Buffer to each tube as described in Table 1. We recommend that you store these diluted standards for no more than one hour.

Tube	1 mM L-Theanine Standard (μ l)	Diluted Assay Buffer (μ l)	L-Theanine Concentration (μ M)
A	0	1,000	0
B	2.5	997.5	2.5
C	5	995	5
D	10	990	10
E	20	980	20
F	30	970	30
G	40	960	40
H	50	950	50

Table 1. Preparation of L-Theanine standard curve

Performing the Assay

1. **Standard Wells** - Add 50 µl of standard (tubes A-H) per well in the designated wells on the plate (see **Sample Plate Format**, Figure 2, page 9).
2. **Sample Wells** - Add 50 µl of the sample to at least two wells. To obtain reproducible results, the amount of L-Theanine added to the wells should fall within the range of the assay. When necessary, samples should be diluted with the diluted Assay Buffer.
3. Add 50 µl of L-Theanine Initiator (Item No. 700573) to all wells being used.
4. Add 50 µl of L-Theanine Detector (Item No. 700574) to all wells being used.
5. Cover the plate with the plate cover, and incubate for 30 minutes at 25°C.
6. Remove the plate cover and read using an excitation wavelength of 430-440 nm and an emission wavelength of 485-495 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence of the standards. Subtract the fluorescence value of standard A (0 µM) from itself and all other standards and samples. This is the corrected fluorescence (CSF).
2. Plot the corrected fluorescence values (from step 1 above) of each standard as a function of the final concentration of L-Theanine from Table 1. See Figure 3, on page 14, for a typical standard curve.
3. Calculate the L-Theanine concentration of the samples using the equation obtained from the linear regression of the standard curve substituting the corrected sample fluorescence (CSF) for each sample.

L-Theanine Concentration (µM) =

$$\left[\frac{\text{CSF} - (\text{y-intercept})}{\text{Slope}} \right] \times \text{Sample dilution}$$

4.

L-Theanine Concentration (mg) =

$$\frac{(\mu\text{M})(174.2 \text{ g/mol})(\text{vol Liter})}{*1,000}$$

*Conversion from gram to milligram L-Theanine.

Performance Characteristics

Sensitivity:

The limit of detection for the assay is 1.5 µM (±0.5 µM) L-Theanine.

Precision:

When a series of ninety-six plasma measurements were performed on the same day, the intra-assay coefficient of variation was 2.6%. When a series of ninety-six plasma measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 2.2%.

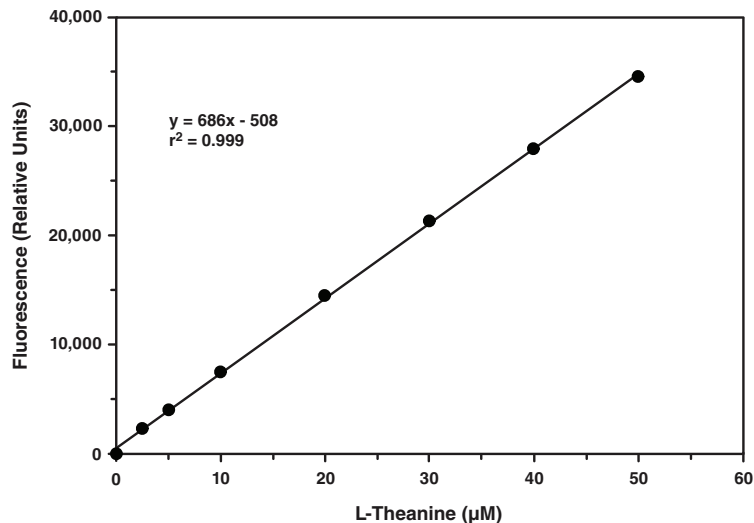


Figure 3. L-Theanine standard curve

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 L-Theanine concentration was obtained above the corrected sample fluorescence (CSF)	A. The L-Theanine concentration is too low to detect B. The sample does not contain L-Theanine	A. Re-assay the sample using a lower dilution
L-Theanine concentration was above the highest point in the standard curve	A. The L-Theanine concentration was too concentrated in the sample	Dilute samples with diluted Assay Buffer and re-assay. <i>NOTE: Remember to account for the dilution factor when calculating L-Theanine concentration.</i>
The fluorometer exhibited "MAX" values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read

References

1. Juneja, L.R., Chu, D.-C., Okubo, T., *et al.* L-theanine-a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Science & Technology* **10(6-7)**, 199-204 (1999).
2. Thippeswamy, R., Gouda, K.G., Roa, D.H., *et al.* Determination of theanine in commercial tea by liquid chromatography with fluorescence and diode array ultraviolet detection. *J. Agric. Food Chem.* **54(19)**, 7014-19 (2006).
3. Eschenauer, G. and Sweet, B.V. Pharmacology and therapeutic uses of theanine. *Am. J. Health-Syst. Pharm.* **63(1)**, 26, 28-30 (2006).
4. Ritsner, M.S., Miodownik, C., Ratner, Y., *et al.* L-theanine relieves positive, activation, and anxiety symptoms in patients with schizophrenia and schizoaffective disorder: An 8-week, randomized, double-blind, placebo-controlled, 2-center study. *J. Clin. Psychiatry* **72(1)**, 34-42 (2011).

Related Products

Ascorbate Assay Kit - Item No. 700420
Catalase Assay Kit - Item No. 707002
Creatinine (serum) Assay Kit - Item No. 700460
Glutathione Assay Kit - Item No. 703002
Glutathione Peroxide Assay Kit - Item No. 703102
Glutathione Reductase Assay Kit - Item No. 703202
Glutathione S-Transferase Assay Kit - Item No. 703302
8-hydroxy-2-deoxy Guanosine EIA Kit - Item No. 589320
Hydrogen Peroxide (urinary) Assay Kit - Item No. 706011
iPF_{2α}-VI EIA Kit - Item No. 516301
8-Isoprostane EIA Kit - Item No. 516351
Lipid Hydroperoxide Assay Kit - Item No. 705002
Protein Carbonyl Assay Kit - Item No. 10005020
Protein Carbonyl Fluorometric Assay Kit - Item No. 700490
Superoxide Dismutase Assay Kit - Item No. 706002
TBARS Assay Kit - Item No. 10009055
Thioredoxin Reductase Assay Kit - Item No. 10007892
Uric Acid Assay Kit - Item No. 700320
Xanthine Oxidase Assay Kit - Item No. 10010895

NOTES

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