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# ab138883 Glutamate Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of glutamate (glutamic acid) in various samples.

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

# 1. Overview

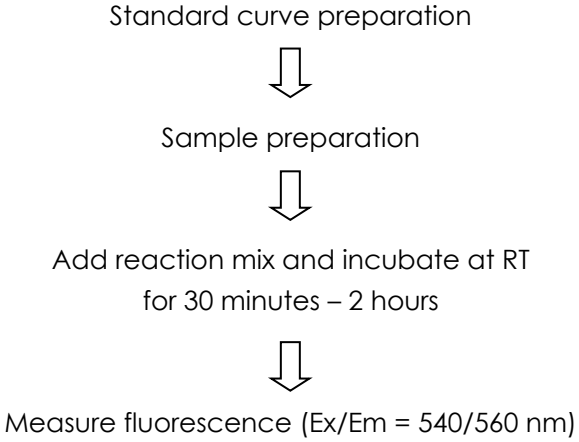
Glutamate Assay Kit (Fluorometric) (ab138883) provides a quick and sensitive method for the measurement of glutamate (glutamic acid) in various biological samples. In this assay, the coupled enzyme system catalyzes the reaction between L-Glutamic acid and $NADP^+$ to produce NADPH, which is specifically recognized by the NADPH sensor and recycled back to $NADP^+$. During the reaction, a red fluorescence product is produced, which in turn can be detected in a fluorescence microplate reader at Ex/Em = 540/590 nm (range Ex/Em = 530 – 570/590 – 600 nm). This assay can detect as little as 1 µM glutamic acid.

The signal can also be read by absorbance at OD: 576 ± 5 nm, although the sensitivity of the assay is reduced 10-fold.

The assay is robust, and can be easily adapted to automation without separation step as no wash step is required.

Glutamate (glutamic acid) is one of the 20 proteinogenic amino acids. The carboxylate anions and salts of glutamic acid are known as glutamates. Glutamate is an important neurotransmitter which plays a key role in long-term potentiation and is important for learning and memory. Glutamic acid is the precursor of GABA but has somewhat the opposite function; it might play a role in the normal function of the heart and the prostate. As one of the few nutrients that crosses the blood-brain barrier, Glutamic acid is used in the treatment of diseases such as depression, ADD and ADHD, fatigue, alcoholism, epilepsy, muscular dystrophy, mental retardation, and schizophrenia.

## 2. Protocol Summary



## 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 4. Storage and Stability

**Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item | Quantity | Storage Condition (before prep) | Storage Condition (after prep) |
|---|---|---|---|
| Assay Buffer | 10 mL | -20°C | -20°C |
| Dilution Buffer | 10 mL | -20°C | -20°C |
| Enzyme Mixture (lyophilized) | 1 bottle | -20°C | -20°C |
| Glutamic Acid | 1 vial | -20°C | -20°C |
| NADP | 1 vial | -20°C | -20°C |

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 540/590 nm (Ex/Em = 530-570/590-600 nm).
- MilliQ water or other type of double distilled water ($ddH_2O$)
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably black
- Dounce homogenizer (if using tissue)
- Cell lysis buffer: we recommend Mammalian Cell Lysis Buffer 5X (ab179835)
- (Optional) BCA Protein Quantification Kit (ab102536) – to determine protein concentration of tissue sample.

## 8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

# 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Dilution Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 **Enzyme Mixture:**

To use immediately add 10 mL of Assay Buffer (Step 9.1) to the bottle of enzyme mixture and mix well by inversion. Label this as **Assay Reaction Mixture**.

**NOTE:** this Assay Reaction Mixture is unstable at room temperature and cannot be stored at- 20°C. It should be used promptly within 2 hours and avoid exposure to light.

**Alternatively**, to store for future use, make a 50X of Enzyme Mixture stock solution by adding 200 µL of H2O into the bottle of enzyme mixture. Aliquot reconstituted mixture so that you have enough to perform the desired number of assays. The 50X Enzyme mix stock can be store at - 20°C. avoid freeze and thaw.

The aliquots of Stock solution can then be mixed with Assay Buffer proportionally.

9.4 **Glutamic Acid:**

Prepare a 100 mM Glutamic Acid Stock Solution by adding 200 µL of Dilution Buffer (Step 9.2) to the vial of Glutamic Acid. Mix well by pipetting up and down. Aliquot Stock solution so that you have enough volume to perform the desired number of assays, Store at -20°C protected from light.

9.5 **NADP:**

Prepare a NADP Stock Solution (200X) by adding 100 µL of Dilution Buffer (Step 9.2) to the vial of NADP. Mix well by pipetting up and down. Aliquot Stock solution so that you have enough volume to perform the desired number of assays, Store at -20°C protected from light.

## 10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Dilute 10 µL of 100 mM Glutamic Acid stock solution (Step 9.4) into 990 µL Dilution Buffer (Step 9.2) to generate a 1000 µM Glutamic Acid standard solution.

10.2 Using 1000 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| Standard # | Sample to dilute | Volume standard in well (µL) | Dilution Buffer (µL) | End conc Glutamic acid in well |
|---|---|---|---|---|
| 1 | 150 µL (from 1000 µM stock) | 100 | 0 | 1000 µM |
| 2 | 50 µL (from Standard #1) | 100 | 100 | 333.33 µM |
| 3 | 50 µL (from Standard #2) | 100 | 100 | 111.11 µM |
| 4 | 50 µL (from Standard #3) | 100 | 100 | 37.04 µM |
| 5 | 50 µL (from Standard #4) | 100 | 100 | 12.35 µM |
| 6 | 50 µL (from Standard #5) | 100 | 100 | 4.12 µM |
| 7 | 50 µL (from Standard #6) | 100 | 100 | 1.37 µM |
| Blank | 0 | 100 | 100 | 0 µM |

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

# 11. Sample Preparation

## General sample information:

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- The preparation step described in this section uses Mammalian Cell Lysis Buffer 5X (ab179835). Other general lysis buffer can be used as long as they don't contain Tween-20, or contain <0.1% SDS or <0.1% NP-40.

## 11.1 Adherent cell samples:

11.1.1 Grow and treat cells as required in your desired culture vessel to about ~80% confluence.

11.1.2 Prepare Mammalian Cell Lysis Buffer 5X (ab179835) as per the product instructions, to obtain 1X Mammalian Lysis Buffer.

11.1.3 Wash cells in PBS to remove residual media.

11.1.4 Lysis cells with 1X Mammalian Lysis Buffer. Table below indicates suggested volumes.

| Plate | 384-wp | 96-wp | 48-wp | 24-wp | 12-wp | 6-wp | 100 $cm^2$ |
|---|---|---|---|---|---|---|---|
| Volume | 20 µL | 50 µL | 100 µL | 150 µL | 200 µL | 300 µL | 800 µL |

11.1.5 Incubate cells at room temperature for 10 – 20 minutes.

11.1.6 You can use the cell lysate directly, Alternatively, centrifuge lysate at 1500 rpm for 5 minutes.

11.1.7 Keep on ice.

## 11.2 Suspension cell samples:

11.2.1 Prepare Mammalian Cell Lysis Buffer 5X (ab179835) as per the product instructions, to obtain 1X Mammalian Lysis Buffer.

11.2.2 Wash cells in PBS to remove residual media.

11.2.3 Add 100 µL of 1X Mammalian Lysis Buffer to 1 – 5 x $10^6$ cells.

11.2.4 Incubate cells at room temperature for 10 – 20 minutes.
11.2.5 Centrifuge lysate at 1500 rpm for 5 minutes.
11.2.6 Transfer supernatant to a new tube.
11.2.7 Keep on ice.

### 11.3 Tissue samples:

11.3.1 Prepare Mammalian Cell Lysis Buffer 5X (ab179835) as per the product instructions, to obtain 1X Mammalian Lysis Buffer.
11.3.2 Harvest 20 – 200 mg tissue.
11.3.3 Wash tissue with cold PBS.
11.3.4 Homogenize tissue with 400 µL of 1X Mammalian Lysis Buffer. Homogenization can be done using a Dounce homogenizer or pestle, sitting on ice, with 10 – 15 passes.
11.3.5 Centrifuge at 2500 rpm for 5 – 10 minutes.
11.3.6 Transfer supernatant to a new tube.
11.3.7 Keep on ice.
11.3.8 Determine protein concentration of your sample.

### 11.4 Plasma, Serum and Urine (and other biological fluids):

Samples can be used directly or diluted in Dilution Buffer for testing.

**Δ Note**: We suggest using different volumes of samples diluted in Dilution Buffer to ensure readings are within the standard curve range.

# 12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

## 12.1 Assay set up:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Dilution Buffer).
- Blank control = 50 µL Dilution Buffer

See example plate layout for assay in the table below.

- BL: Blank control.
- GLU: Glutamic acid standards.
- TS: test sample wells.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BL | BL | TS | TS | …. | …. | … | …. |
| GLU1 | GLU 1 | …. | …. | …. | …. | …. | …. |
| GLU 2 | GLU 2 | | | | | | |
| GLU 3 | GLU 3 | | | | | | |
| GLU 4 | GLU 4 | | | | | | |
| GLU 5 | GLU 5 | | | | | | |
| GLU 6 | GLU 6 | | | | | | |
| GLU 7 | GLU 7 | | | | | | |

## 12.2 Glutamic acid assay procedure:

12.2.1 Glutamic acid Reaction Mix preparation: add 50 µL (or proportional volume) of 200X NADP Stock Solution (Step 9.5) into the Assay Reaction Mixture (Step 9.3) and mix well by inversion.

Δ **Note**: This Glutamic acid reaction mix is enough for 2 x 96-well plates. It is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light.

12.2.2 Add 50 µL of Reaction Mix into each blank control, standard and sample wells.

12.2.3 Mix and incubate at room temperature for 30 min – 2 hours, protected from light.

12.2.4 Measure fluorescence increase on a microplate reader at Ex/Em = 540/590 nm.

Δ **Note:** This assay can also be done on a white clear bottom plate and read by a colorimetric microplate reader at wavelength OD = 576 ± 5 nm. The absorption detection has lower sensitivity (approximate 10-fold) compared to fluorescence detection.

Δ **Note:** If reading at specific time points does not look linear, we recommend that you monitor fluorescence increase using kinetic mode instead of end-point mode.

## 13. Protocol for 384-well plate assay

13.1 Prepare standard as described in Section 10.
13.2 Prepare samples as described in Section 11.
13.3 Assay set up: add 25 µL standard dilutions, 25 µL test samples and 25 µL blank control.
13.4 Prepare Glutamic acid reaction mix as described in Section 12.2.1.
13.5 Add 25 µL reaction mix into each well.
13.6 Mix and incubate at room temperature for 30 min – 2 hours, protected from light.
13.7 Measure fluorescence increase on a microplate reader at Ex/Em = 540/590 nm.

# 14. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

14.1 Subtract the mean fluorescence value of the blank (BL) from all standard and sample readings for each data point. This is the corrected fluorescence.

14.2 Average the duplicate reading for each standard and each of the test samples.

14.3 Plot the corrected fluorescence values for each standard as a function of the final concentration of NADPH.

14.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

14.5 Concentration of Glutamic Acid in the test samples is calculated as:

$$\text{Glutamic acid concentration} = \left(\frac{B}{V}\right) * D$$

Where:

B = Glutamic acid amount in the sample well calculated from standard curve (µM).

V = sample volume added in the sample wells (µL).

D = sample dilution factor.

# 15. Typical Data

**Typical standard curve** – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

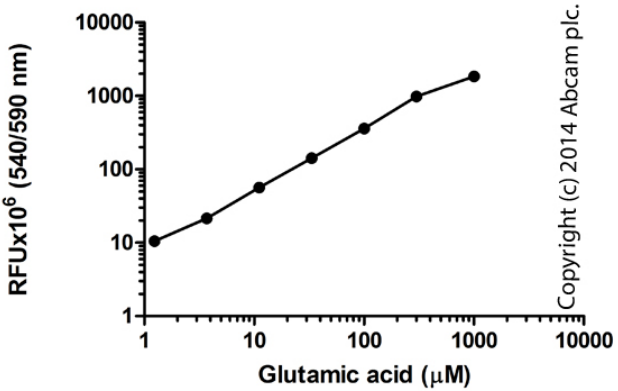



**Figure 1**. Typical Glutamic Acid standard calibration curve. Assay was measured in a black 96-well plate after 30 minute incubation. Mean of duplicated (± SD), with background reads subtracted.

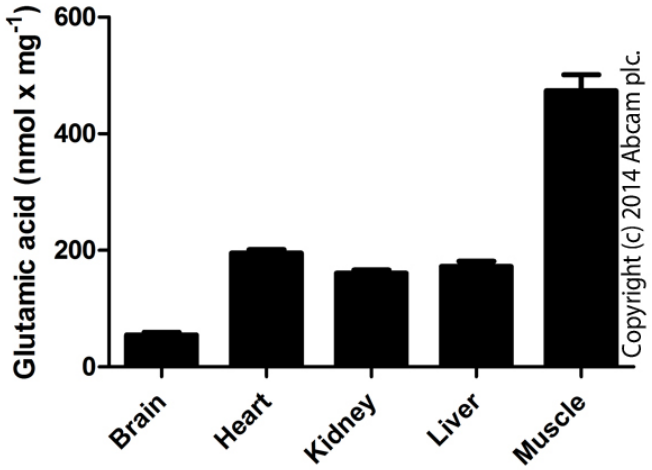



**Figure 2**. Glutamic Acid measured in mouse tissue lysates, showing quantity (nmol) per mg of extracted protein (mean of duplicates, ± SD).

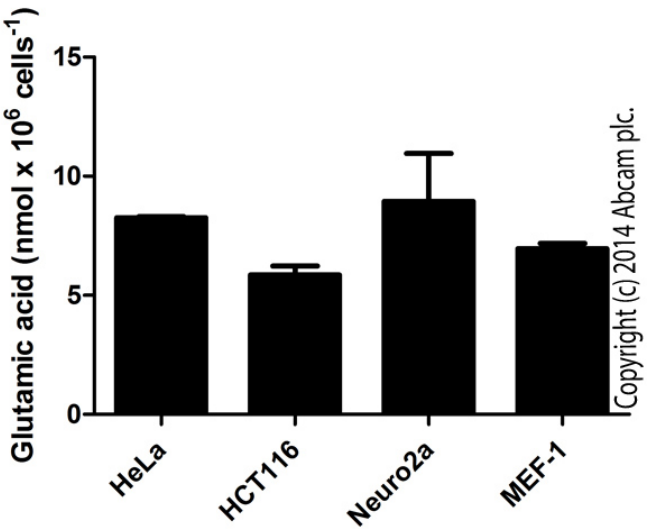



Figure 3. Glutamic Acid measured in cell lysates, showing quantity (nmol) per $10^6$ cells (mean of duplicates, ± SD).

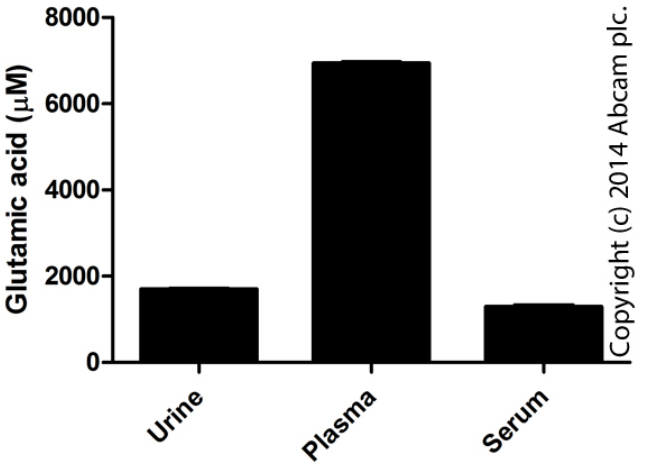



Figure 3. Glutamic Acid measured human biological fluids (mean of duplicates, ± SD).

## 16. Quick Assay Procedure

**Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.**

- Solubilize Glutamic Acid standard, prepare Assay Reaction Mixture and NADP Stock solution (aliquot if necessary); get equipment ready.
- Prepare Glutamic Acid standard dilution [300 – 1 µM].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL) and blank control (50 µL).
- Prepare Glutamic acid reaction mix by adding 50 µL of NADP 200X Stock to Assay Reaction Mixture.
- Add 50 µL Reaction to each well.
- Incubate plate at RT for 30 minutes – 2 hours protected from light.
- Monitor fluorescence increase in a fluorescence microplate reader at Ex/Em= 540/590 nm.

## 17. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Assay not working | Use of ice-cold buffer | Buffers must be at assay temperature |
| | Plate read at incorrect wavelength | Check the wavelength and filter settings of instrument |
| | Use of a different microplate | Colorimetric: clear plates<br>Fluorometric: black wells/clear bottom plates |
| Sample with erratic readings | Cells/tissue samples not homogenized completely | Use Dounce homogenizer, increase number of strokes |
| | Samples used after multiple free/ thaw cycles | Aliquot and freeze samples if needed to use multiple times |
| | Use of old or inappropriately stored samples | Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use |
| | Presence of interfering substance in the sample | Check protocol for interfering substances; deproteinize samples |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Allowing reagents to sit for extended times on ice | Always thaw and prepare fresh reaction mix before use |
| | Incorrect incubation times or temperatures | Verify correct incubation times and temperatures in protocol |

| Problem | Reason | Solution |
|---|---|---|
| **Standard readings do not follow a linear pattern** | Pipetting errors in standard or reaction mix | Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the tubes |
| | Standard stock is at incorrect concentration | Always refer to dilutions described in the protocol |
| **Unanticipated results** | Measured at incorrect wavelength | Check equipment and filter setting |
| | Samples contain interfering substances | Troubleshoot if it interferes with the kit |
| | Sample readings above/ below the linear range | Concentrate/ Dilute sample so it is within the linear range |

## 18. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Phenol red: will interfere with absorbance reading, but will have no effect on fluorescent reading.
- Antioxidant molecules such as cysteineamide: will reduce the developer and will give false positive results.

## 19. FAQs

### Q. Do I need to deproteinize my sample to use this assay?

A. No, is not necessary. Although sample deproteinization is recommended for our colorimetric assay Glutamate Assay Kit (ab83389), the working principle for this product is different and therefore is not necessary to deproteinize sample.

# 20. Notes

## Technical Support



**For all technical or commercial enquiries please go to:**

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)