Version 6b, updated 12 June 2025

ab252893
Glutamate Assay Kit (Fluorometric)

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For the measurement of Glutamate in various biological samples/preparations.

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

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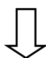
1. Overview

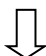
Glutamate Assay Kit (Fluorometric) (ab252893) allows for quantification of Glutamate in biological fluids and tissues. The assay is based on an enzymatic reaction in which a fluorogenic probe is reduced producing a stable signal. The reduced fluorophore produces a strong signal (Ex/Em= 535/587 nm), which is directly proportional to the amount of Glutamate in samples. The assay is simple, reproducible, and can specifically detect as low as 5 pmol of Glutamate in a 100 µL reaction.

2. Protocol Summary

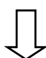
Prepare Samples, Standards and Background controls as directed.



Prepare reaction master mix



Add Samples, Standards and Background controls (50 µL) to Reaction Mix (50 µL) in appropriate wells of a 96-well, white, flat-bottomed plate.



Measure fluorescence at Ex/Em = 535/587 in kinetic mode for 60 mins at 37°C.

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Glutamate Assay Buffer	50 mL	-20°C	-20°C
Enzyme Mix VIII	1 vial	-20°C	-20°C
Glutamate Standard	0.1 mL	-20°C	-20°C
Developer Mix T	1 vial	-20°C	-20°C
PicoProbe I	0.4 mL	-20°C	-20°C

PLEASE NOTE: Enzyme Mix VIII was previously labelled as Glutamate Enzyme Mix, and PicoProbe I as Probe (in DMSO), and Developer Mix T as Developer XI and Glutamate Substrate Mix. The composition has not changed.

4. Materials Required, Not Supplied

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

– Fluorescence microplate reader capable of reading at Ex/Em = 535/587 nm.
– 96-well white plate with flat bottom.
– Dounce homogenizer.

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Glutamate Assay Buffer

Ready to use as supplied. Store at -20 °C. Bring to 37 °C before use.

6.2 Enzyme Mix VIII

Reconstitute with 220 µL of Glutamate Assay Buffer and mix thoroughly. Store at -20 °C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.

6.3 Glutamate Standard

Ready to use as supplied. Store at -20 °C.

Developer Mix T

Reconstitute with 220 µL of Glutamate Assay Buffer and mix thoroughly. Store at -20 °C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.

6.4 PicoProbe I

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

7. Standard Preparation

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

7.1 Prepare a 1 mM Glutamate Standard by adding 5 µL of Glutamate Standard/0.1 M Glutamate Standard to 495 µL Glutamate Assay Buffer, mix well; further prepare a 10 µM of Glutamate Standard by adding 5 µL of 1 mM Glutamate Standard to 495 µL Glutamate Assay Buffer, mix well.

7.2 Add 0, 2, 4, 6, 8, 10 µL of 10 µM (10 pmol/µL) Glutamate standard into a series of wells to generate 0, 20, 40, 60, 80, 100 pmol of Glutamate/well respectively. Adjust the volume to 50 µL/well with Glutamate Assay Buffer.

Standard #	Glutamate 10 pmol/ µL Standard (µL)	Glutamate Assay Buffer (µL)	Final volume standard in well (µL)	End amount Glutamate in well (pmol/well)
1	0	100	50	0
2	4	96	50	20
3	8	92	50	40
4	12	88	50	60
5	16	84	50	80
6	20	80	50	100

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

8. Sample Preparation

General sample information:

– We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

– 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.

1

8.1 Tissue and cell lysates:

8.1.1 Homogenize tissue (10~20 mg) or pelleted cells (~1 x 10^6) with 400 µL ice-cold Glutamate Assay Buffer and keep on ice for 10 mins.

8.1.2 Centrifuge samples at 12,000 *x g* at 4°C for 10 mins and collect the supernatant.

Δ **Note:** If a high concentration of glutamate is expected, dilute the supernatant 10-50 fold in Glutamate Assay Buffer.

8.1.3 Add 2-10 µL of Diluted Sample(s) into well(s) of a 96-well white plate.

8.2 Serum and cerebrospinal fluid:

8.2.1 Clarify samples by centrifugation at 10,000 *x g* at 4°C for 5 mins in order to reduce turbidity and separate insoluble material.

8.2.2 Prepare a 10-fold dilution of serum in dH_2O (such as add 10 µL of serum with 90 µL of dH_2O). CSF can be added directly.

8.2.3 Add 2-10 µl of sample (CSF or diluted serum) into well(s) of a 96-well white plate. Adjust the volume of Sample(s) to 50 µL/well with Glutamate Assay Buffer.

Δ **Note:** We suggest using 3-5 different amounts of unknown samples to ensure the readings are within the standard curve range and the changes of velocity are within the linear range.

9. 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.

9.1 Reaction mix preparation:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µL Mix containing:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Glutamate Assay Buffer	45	47
Enzyme Mix VIII	2	0
Developer Mix T	2	2
PicoProbe I	1	1

2. Mix and add 50 µL of the Reaction Mix to each well containing the Glutamate Standard(s) and Sample(s). Mix and add 50 µL of Background Reaction Mix to Sample Background Control wells. The total final reaction volume for each well will be 100 µL.

Δ Note: Sample background control should be set up for each sample to account for sample constituents that might contribute to false positive signal.

9.2 Measurement:

1. Measure fluorescence intensity (Ex/Em= 535/587 nm) in kinetic mode at 37°C for 60 mins using a fluorescence microtiter plate reader. Choose two time points (t1 & t2) in the linear range of

the plot and obtain the corresponding RFU for all Samples (RS1 and RS2) and Sample Background Controls (RB1 and RB2).

9.3 Calculation:

1. Plot a standard curve in End-point mode:

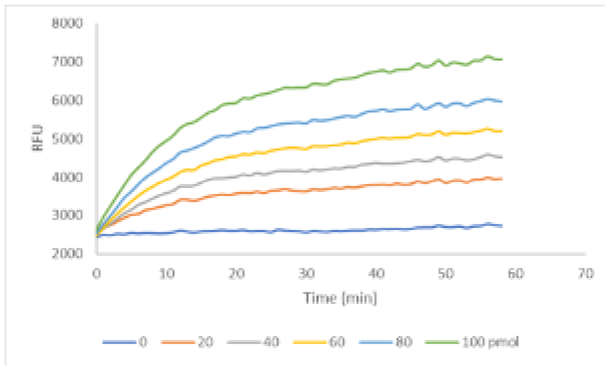


Figure 1. Exemplary data for the standard curve measurement (Ex/Em= 535/587 nm) in kinetic mode at 37°C for 60 mins.

a. Select two time points at the end of the curves where the kinetics of the reactions are linear (t1 and t2). Extrapolate the linear portion between t1 and t2 for each curve to obtain the Y-intercept (y-value at x = 0).

Δ Note: It is normal to observe drifting in Glutamate Standards.

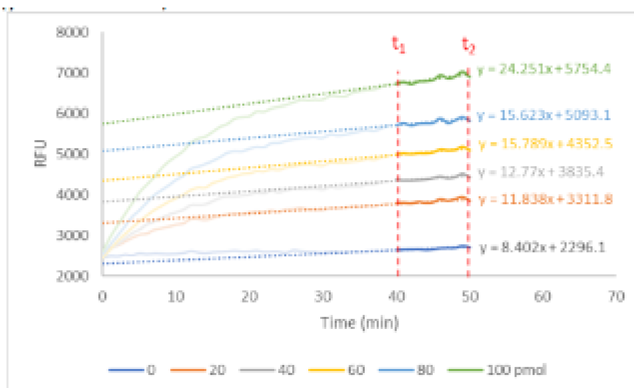


Figure 2. Exemplary depiction of defining a linear range (t1 and t2) towards the end of the curves.

b. Subtract the y-intercept for 0 pmole glutamate (the reagent blank) from all other standard curve intercept to generate the corrected y-intercept values.
c. If the sample background control is significant, then subtract the sample background control from sample reading.
d. Plot the Standard Curve using the corrected Y-intercept values and calculate the linear regression that best fits the data.

2. Extrapolate the linear portion for your sample measurements and calculate the glutamate amount:
   A. Using the same time points selected for the standard curve, extrapolate the linear portion between t1 and t2 for each curve from your sample measurements to obtain the Y-intercept (y-value x = 0).

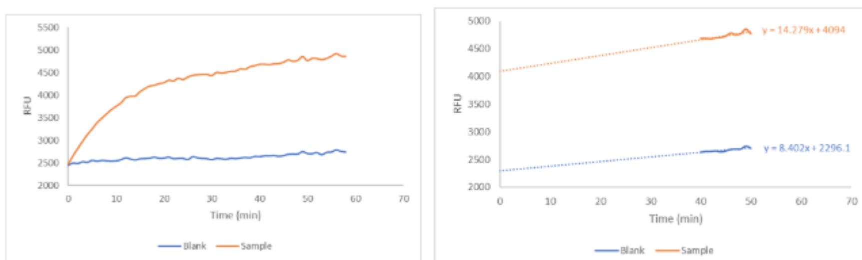


Figure 3. Depiction of two exemplary sample measurements (left) and application of the defined linear range (t1 and t2) on the measurement (right).

   B. Subtract the Y-intercept for the blank (background) from the sample data to generate the corrected Y-intercept.

C. Calculate the B value, the amount of glutamate (in pmoles) in the sample using the equation of the linear regression from the standard curve and the Corrected Y-intercept.
D. Considering the volume of the sample the concentration of glutamate can be calculated.

Using the concentration of protein in the sample (from BCA assay), the amount of glutamate per mg of protein can be calculated:

Sample Glutamate amount = B/(V x P) x D = pmol/mg

Where:
B = Glutamate amount (Step 2D)
V = sample volume added into the reaction well (mL)
P = initial Sample protein concentration (mg/mL)
D = Sample dilution factor

10. Typical Data

Data provided **for demonstration purposes only**.

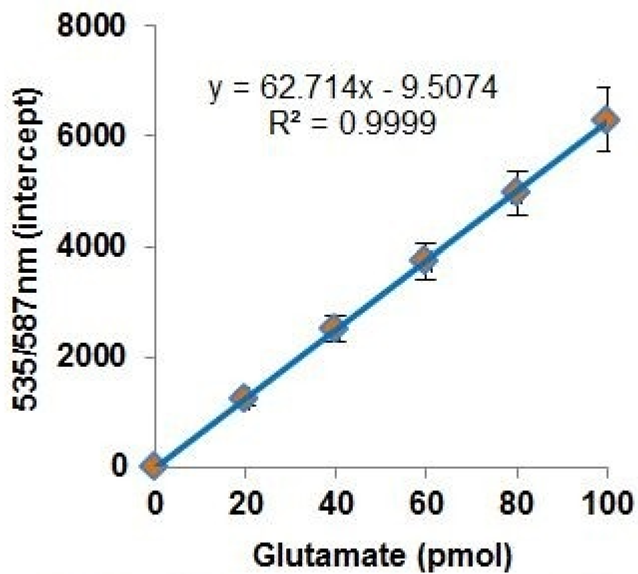


Figure 4. Glutamate standard curve. Data from multiple experiments.

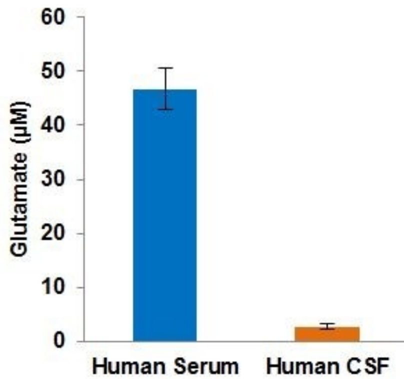


Figure 5. Measurement of Glutamate amounts**.** Pooled normal human serum (1:10; 5 µL) and pooled normal human CSF (5 µL; undiluted).

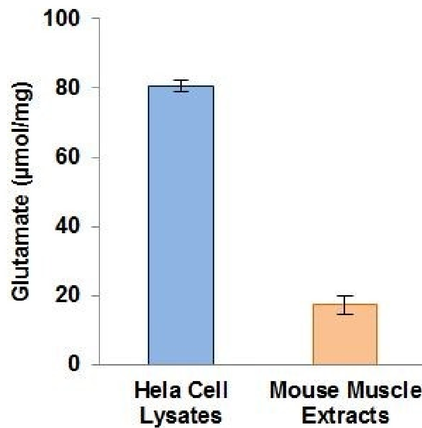


Figure 6. Measurement of Glutamate**.** HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysates (0.5 µg protein) and mouse muscle extracts (0.5 µg protein).

11. Notes

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