



PicoProbe[™] Glutamate Carboxypeptidase II Activity Assay Kit

(Catalog # K738-100; 100 assays; Store at -20°C)

I. Introduction:

Glutamate Carboxypeptidase II [GCPII, EC 3.4.17.21; *N*-acetylated-alpha-linked acidic dipeptidase (NAALADase), prostate-specific membrane antigen (PSMA) or folate hydrolase (FOLH1)] is a zinc-metallopeptidase, multifunctional protein. In humans, it is mainly expressed in the nervous system, prostate, small intestine and kidney. In males, PSMA is overexpressed in patients suffering prostate cancer and thus, it is used as a diagnostic marker for this disease. NAALADase, the brain isozyme, plays an important role in Glutamate biosynthesis and could be used for treating neuronal damage caused by excess glutamate in brain. BioVision's PicoProbe[™] Glutamate Carboxypeptidase II Activity Assay Kit is a simple plate-based fluorometric assay for the measurement of GCPII activity in Biological Samples. The provided substrate is transaminated in the presence of GCPII producing glutamate. The detection system is based on an enzymatic reaction in which a fluorogenic probe is reduced generating a stable signal. The reduced fluorophore produces a strong signal which is directly proportional to the amount of active GCPII in samples. The assay can detect as low as 0.5 µU of Glutamate Carboxypeptidase II.

GCPII Substrate GCPII Enzyme Mix + GCPII Developer Probe
Fluorescent Product (Ex/Em = 535/587 nm)

II. Applications:

• Measurement of Glutamate Carboxypeptidase II Activity in Biological Samples/Preparations.

III. Sample Type:

- Tissue Homogenates: Mouse Kidney, etc.
- Purified Enzyme or protein preparations.

IV. Kit Contents:

Components	K738-100	Cap Code	Part Number
GCPII Assay Buffer	35 ml	NM	K738-100-1
PicoProbe [™] (in DMSO)	0.4 ml	Blue	K738-100-2
GCPII Substrate	35 µl	Orange	K738-100-3
GCPII Positive Control	1 vial	Purple	K738-100-4
GCPII Enzyme Mix	1 vial	Green	K738-100-5
GCPII Developer	1 vial	Red	K738-100-6
Glutamate Standard (0.1 M)	0.1 ml	Yellow	K738-100-7

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (Fluorescent plate reader)
- 96-well white plate with flat bottom
- Dounce Tissue Homogenizer (Cat. #1998)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- GCPII Assay Buffer: Store at either 4 °C or -20 °C. Bring to 37 °C before use.
- PicoProbe[™]: Ready to use as supplied. Warm to room temperature before use. Store at -20 °C.
- GCPII Substrate: Ready to use as supplied. Store at -20 °C.
- GCPII Positive Control: Reconstitute with 20 μl of GCPII Assay Buffer. Pipette up and down to mix well. Aliquot and store at -20 °C. Keep on ice while in use. Use in two months.
- GCPII Enzyme Mix: Reconstitute with 220 µl of GCPII Assay Buffer and mix thoroughly. Aliquot and store at -20 °C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.
- GCPII Developer: Reconstitute with 220 µl of GCPII Assay Buffer and mix thoroughly. Aliquot and store at -20 °C.
- Glutamate Standard: Ready to use as supplied. Store at -20 °C.

VII. Glutamate Carboxypeptidase II Assay Protocol:

1. Sample Preparation: For Tissue: Homogenize tissue (~50 mg) with 200 µl ice-cold GCPII Assay Buffer and keep on ice for 10 min. Centrifuge samples at 12,000 x g and 4 °C for 15 min and collect the supernatant. Estimate protein concentration using preferred method. We recommend BCA protein assay kit (BioVision: Cat# K813-2500). Protein concentration should range between 4-10 µg/µl. Remove endogenous interference from tissues by using ammonium sulfate: Aliquot samples (100 µl) to clean centrifuge tubes, add same volume of saturated ammonium sulfate (~4.1 M; RT), set on ice for 30 min., mix well, and spin down at 14,000 x g and 4°C for 5 min. (Do not vortex), discard the supernatant. Repeat the same procedure one more time and resuspend the pellet to the original volume using GCPII Assay Buffer. Dilute treated samples 10-fold. Prepare duplicate wells by adding 2-20 µl of Diluted Samples into wells of a 96-well white plate (labeled as "Sample" and "Sample Background Control"). For Positive Control: Prepare a 10-fold dilution of GCPII Positive Control Stock solution (i.e. 2 µl of GCPII Positive Control stock solution + 18 µl GCPII Assay Buffer). Add 4-10 µl of Diluted GCPII Positive Control into well(s) as Positive Control. Adjust the volume of Positive Control, Sample wells, and Sample Background Control to 50 µl/well with GCPII Assay Buffer.



Note:

- a) Endogenous metabolites interfere with the assay. Samples must be treated with Ammonia Sulfate.
- b) We suggest using 3-5 different amounts of the samples per well to ensure the readings are within the standard curve range and rates (progress curves kinetics) are within the linear range.
- 2. Standard Curve Preparation: Prepare a 1 mM Glutamate Standard by adding 2 μl of 0.1 M Glutamate Standard to 198 μl GCPII Assay Buffer; Further dilute to 10 μM of Glutamate Standard by adding 2 μl of 1 mM Glutamate Standard to 198 μl Glutamate Assay Buffer. Add 0, 2, 4, 6, 8, 10 μl of 10 μM (10 pmol/μl) of Glutamate standard into a series of wells of 96-well white plate to generate 0, 20, 40, 60, 80, 100 pmol of Glutamate/well respectively. Adjust the volume to 50 μl/well with GCPII Assay Buffer.
- 3. Reaction Mix Preparation: Prepare a 100-fold dilution of GCPII Substrate (2 µl of GCPII Substrate + 198 µl GCPII Assay Buffer). Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Reaction Mix	Background Mix
GCPII Assay Buffer	35 µl	45 µl
Diluted GCPII Substrate	10 µl	
GCPII Enzyme Mix	2 µl	2 µl
GCPII Developer	2 µl	2 µl
PicoProbe [™]	1 µl	1 µl

Mix well and add 50 µl of the Reaction Mix to well(s) containing Glutamate Standard(s), Sample(s) and Positive Control. Add 50 µl of Background Mix to well(s) containing Sample Background Control, mix well. *Final Volume: 100 µl*.

Note: Prepare Reaction Mix immediately before adding to wells. Do not store unused Reaction Mix.

- 4. Measurement: Immediately measure fluorescence intensity (Ex/Em = 535/587 nm) in kinetic mode at 37°C for 60-90 min using a fluorescence microtiter plate reader. Standards may be read in either kinetic or end point mode (after 60 min). For samples, choose two time points (t₁ & t₂) in the linear range of the plot and obtain the corresponding RFU for Sample (R_{S1} and R_{S2}) and Sample Background Control (R_{B1} and R_{B2}). Note: It is normal to observe a lag phase in Positive Control and Samples within the first 30 min. Linear ranges are usually observed after 30 minutes of reaction.
- 5. Calculation: Subtract 0 Standard Reading from all Standard(s) Readings. Plot the Glutamate Standard Curve and obtain the slope of the curve (RFU/pmol). Apply Sample △RFU and Sample Background Control △RFU to Glutamate Standard Curve to obtain the corresponding amount of Glutamate formed during the reaction time (△t = t₂-t₁). Calculate the background-corrected sample △RFU (B, in pmol) by subtracting the amount of Glutamate formed by Sample Background Control from the amount of Glutamate formed by Sample. Calculate Glutamate Carboxypeptidase II activity in the sample as:

Sample GCPII Activity = (B test sample-B Sample Background control) * D= pmol/min/mg = µU/mg

 $\Delta t \cdot V.P$

Where: **B** is Glutamate **amount** from Standard Curve (pmol)

- Δt is Reaction time (t₂-t₁, min)
- V is Sample volume added into the reaction well (ml)
- **P** is Initial **protein** sample Concentration in mg-protein/ml (mg/ml)
- D is Dilution factor

Unit Definition: One unit of GCPII activity is the amount of enzyme that generates 1.0 µmol of Glutamate per min at pH 7.4 at 37 °C.



Figure: (a) Glutamate Standard Curve, results from multiple experiments. (b) Purified Glutamate Carboxypeptidase II activity and GCPII activity in Mouse Kidney. (c) Measurement of GCPII activity in Mouse Kidney Extracts (6 µg protein). All assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Glutamate Carboxypeptidase II Inhibitor Screening Kit (KXXX) Glutamate Colorimetric Assay Kit (K629) Human CellExpTM PSMA/FOLH1, Human Recombinant (P1304) PicoProbeTM Glutamate Assay Kit (Fluorometric) (K413)

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