



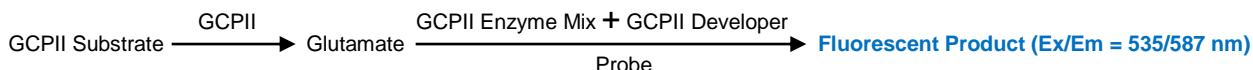
PicoProbe™ Glutamate Carboxypeptidase II Activity Assay Kit

1/19

(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-metalloprotein, 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.



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.

