ab284558 – Glutamate Carboxypeptidase II Activity Assay Kit

For the detection of Glutamate Carboxypeptidase II (GCPII) activity in biological samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284558

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
GCPII Assay Buffer	35 mL	-20°C
GCPII Developer	1 vial	-20°C
GCPII Enzyme Mix	1 vial	-20°C
GCPII Positive Control	1 vial	-20°C
GCPII Substrate	35 µL	-20°C
Glutamate Standard (0.1 M)	0.1 mL	-20°C
PicoProbe (in DMSO)	0.4 mL	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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.

Assay Protocol

Sample preparations:

1. **For Tissue**: Homogenize tissue (~50 mg) with 200 µL ice-cold GCPII Assay Buffer and keep on ice for 10 min.
2. Centrifuge samples at 12,000 x g and 4°C for 15 min and collect the supernatant.
3. Estimate protein concentration using preferred method.
4. Protein concentration should range between 4-10 µg/µL.
5. 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.
6. Repeat the same procedure one more time and resuspend the pellet to the original volume using GCPII Assay Buffer.
7. 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").
8. **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).
9. Add 4-10 µL of Diluted GCPII Positive Control into well(s) as Positive Control.
10. Adjust the volume of Positive Control, Sample wells, and Sample Background Control to 50 µL/well with GCPII Assay Buffer.

Δ Notes:
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.*

Standard Curve Preparation:

1. 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.
2. 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.
3. Adjust the volume to 50 µL/well with GCPII Assay Buffer.

Reaction Mix Preparation:

1. Prepare a 100-fold dilution of GCPII Substrate (2 µL of GCPII Substrate + 198 µL GCPII Assay Buffer).
2. 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

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

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

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 (t_1 & t_2) in the linear range of the plot and obtain the corresponding RFU for Sample (RS_1 and RS_2) and Sample Background Control (RB_1 and RB_2).

Δ 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.*

Calculation:

1. Subtract 0 Standard Reading from all Standard(s) Readings.
2. Plot the Glutamate Standard Curve and obtain the slope of the curve (RFU/pmol).
3. Apply Sample ΔRFU and Sample Background Control ΔRFU to Glutamate Standard Curve to obtain the corresponding amount of Glutamate formed during the reaction time ($\Delta t = t_2 - t_1$).
4. Calculate the background-corrected sample ΔRFU (B, in pmol) by subtracting the amount of Glutamate formed by Sample Background Control from the amount of Glutamate formed by Sample.
5. Calculate Glutamate Carboxypeptidase II activity in the sample as:

$$\text{Sample GCPII Activity} = \frac{(B\ test\ sample - B\ sample\ background\ control)}{\Delta t \cdot V.P} \times D = pmol/min/mg = \mu U/mg$$

Where:
B = Glutamate amount from Standard Curve (pmol)
Δt = Reaction time (t_2-t_1, min)
V = Sample volume added into the reaction well (mL)
P = Initial protein sample Concentration in mg-protein/ml (mg/mL)
D = 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.

Technical Support

For all technical or commercial enquiries please go to:
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