ab282914 – Glutamate Decarboxylase Activity Assay Kit (Fluorometric)

For the measurement GAD acitivty of pure enzymes and in plant and animal tissues and the mechanistic study of GAD.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab282914

Storage and Stability

Entire assay kit should be stored at -20°C, protected from light for up to 12 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
GAD Assay Buffer	25 ml	-20°C
PicoProbe (in DMSO)	0.4 ml	-20°C
GAD Substrate	400 µl	-20°C
GAD Cofactor	200 µl	-20°C
GAD Converter	200 µl	-20°C
GAD Developer	1 vial	-20°C
GAD Positive Control	1 vial	-20°C
GAD Reconstitution Buffer	100 µl	-20°C
NADPH Standard	1 vial	-20°C

Materials Required, Not Supplied

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

- 96-well white opaque plate with flat bottom
- Multi-well spectrophotometer (fluorescence plate reader)
- Multi-channel pipette
- 10kD Spin Column (ab93349)

Reagent Preparation Before using the kit, spin the tubes prior to opening and bring assay components to room temperature (RT) before use.
GAD Assay Buffer, GAD Substrate & GAD Cofactor: Ready to use. Store at 4°C.
PicoProbe (in DMSO): Thaw the vial at RT and mix well. Store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.
GAD Converter: Ready to use. Store at -20°C.
GAD Developer: Reconstitute the vial with 220 µl of GAD Assay Buffer. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.
Use within two months. Keep on ice while in use.
GAD Positive Control: Reconstitute the vial with 100 µl of GAD Reconstitution Buffer. Keep the reconstituted GAD Positive Control on ice for 10 min. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.
NADPH Standard: Reconstitute the vial with 200 µl of dH2O to generate 1 mM (1 nmol/µl) NADPH Standard solution. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.

Assay Protocol

Sample Preparation

1. Homogenize tissue (~25 mg) with 500 µl ice-cold GAD Assay Buffer. Keep on ice for 10min.
2. Centrifuge at 10,000 x g, 4°C for 20 min and collect the supernatant.
3. Use 10 kDa spin column to remove small interfering molecules.
4. Aliquot 100 µl Sample(s) in a clean centrifuge tube & add 400 µl GAD Assay Buffer. Mix well.
5. Add 100 µl of GAD Assay Buffer into the 10 kDa spin column and spin down at 10,000 x g and 4°C for 2 min.
6. Remove the GAD Assay Buffer completely and load 500 µl of the diluted sample(s) to the upper bucket of the spin column and spin at 10,000 x g and 4°C for 20 min. Discard the flow through.
7. Add an additional 300 µl of GAD Assay buffer to the spin column and spin at 10,000 x g and 4°C for 20 min.
8. Repeat 2 more times and save the Sample ultraconcentrate from the upper bucket (for testing the GAD activity).
9. Bring Sample volume to 100 µl. Dilute the Sample(s) ultraconcentrate 10-fold with GAD assay buffer (10 µl of Sample and 90 µl of GAD Assay Buffer) for the assay.
10. Add 2-50 µl Sample(s) into two parallel wells designated as Sample (S) and Sample Background Control (SBC) of a 96-well white plate.
11. For GAD Positive Control (PS) well, add 2-20 µl of GAD Positive Control into the desired well(s).
12. Adjust the volume of the PS, S and SBC wells to 50 µl/well with GAD Assay Buffer.

Notes:

- For Unknown Samples, we suggest doing a pilot experiment to test several doses of the Sample to ensure that the readings are within the Standard Curve range. Samples can be stored at -20°C.
- Protein concentration in Samples can be determined using BCA Protein Assay Kit (ab207003)

Standard Curve Preparation

1. Dilute 1 mM NADPH Standard 50-fold to 20 µM (20 pmol/µl) by adding 20 µl of 1 mM NADPH Standard solution to 980 µl of dH2O.
2. Add 0, 2, 4, 6, 8, and 10 µl of 20 µM NADPH Standard into a series of wells in a 96-well plate to generate 0, 40, 80, 120, 160 and 200 pmol/well of NADPH Standard.
3. Adjust the volume to 50 µl/well with GAD Assay Buffer.

Reaction Mix Preparation

1. Mix enough reagents for the number of assays to be performed.

Component	Reaction Mix	Background Control Mix
GAD Assay Buffer	38 µl	42 µl
GAD substrate	4 µl	---
GAD Cofactor	2 µl	2 µl
GAD Converter	2 µl	2 µl
PicoProbe	2 µl	2 µl
GAD Developer	2 µl	2 µl

2. Mix and add 50 µl of Reaction Mix into each well containing Standards, PS and S. Mix well. Add 50 µl of Background Control Mix to the SBC well(s).
3. Once a Sample is determined to have insignificant background it can be run without SBC.

Measurement

1. Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-60 min at 37°C.

Note: Incubation time depends on the GAD activity in the Sample(s). We recommend measuring fluorescence in a kinetic mode and choosing any two time points (t1 and t2) in the linear range to calculate the GAD activity of the Sample(s). The NADPH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

Calculation:

1. Subtract 0 Standard reading from all Standard readings.
2. Plot the NADPH Standard Curve.
3. Choose any two time points within the linear portion of the curve (t_1 & t_2) for each Sample type. If the SBC RFU reading is significant, subtract the SBC RFU reading from all Sample RFU readings for the chosen t_1 & t_2 time points.
4. Calculate the GAD activity of the Sample: $\Delta RFU = RFU_2 – RFU_1$. Apply ΔRFU to NADPH Standard Curve to get B pmol of NADPH generated by GAD during the reaction time ($\Delta t = t_2 - t_1$)

Sample GAD Activity = $B/(\Delta t \times V) \times D$ = pmol/min/µl = µU/µl = mU/ml

Where:
B = The NADPH amount in the Sample well from Standard Curve (pmol)
Δ**t** = Reaction time (min)
V = Sample volume added into the reaction well (µl)
D = Dilution factor (D = 1 if undiluted)

GAD Activity in Sample(s) can also be expressed in mU/mg of protein. Unit Definition: One unit of GAD is the amount of enzyme that generates 1.0 µmol of NADPH per min at pH 8.8 at 37°C.

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)

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