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Glutamate Decarboxylase Activity Assay Kit (Fluorometric)

10/19

(Catalog # K2021-100; 100 assays; Store at -20°C)

I. Introduction:

Glutamate Decarboxylase (GAD; EC 4.1.1.15, also called Glutamic Acid Decarboxylase) is a pyridoxal phosphate-dependent enzyme that catalyzes the irreversible decarboxylation of L-Glutamate to carbon dioxide and γ-Aminobutyroc Acid (GABA). GAD plays an important role in plants and mammals. GAD activity is associated with plant viability (as measured by germination) and organ development. In humans, GAD is expressed in the brain and β cells of the pancreas namely GAD_{65} and GAD_{67}. In human brain, GAD is the rate-limiting enzyme for generating GABA, the main inhibitory neurotransmitter of the central nervous system. Alterations in the level of GAD or GABA leads to many neurological disorders such as Parkinson's disease, anxiety and epilepsy. GAD is one of the strongest autoantigen that triggers T-cell-mediated autoimmune diabetes in human pancreas. Recent studies have shown that the administration of adeno-associated virus-GAD gene therapy in Parkinson's patients reduces the symptoms of the disease. Additionally, suppression of GAD expression in β cells results in the prevention of diabetes in mouse model. Thus, the study of GAD can be used to develop novel treatment strategies for neurological disorders and type 1 diabetes. In BioVision's **Glutamate Decarboxylase Activity Assay Kit**, GAD decarboxylases L-Glutamate in the presence of Pyridoxal Phosphate to produce CO_2 and GABA intermediates. The intermediates then go through a series of enzymatic reactions to form NADPH, which converts the non-fluorescent probe to a fluorescent product measured at Ex/Em = 535/587 nm. The activity of GAD is proportional to the fluorescent signal. BioVision's Glutamate Decarboxylase Activity Assay Kit offers a rapid, simple, sensitive, and reliable test for detecting GAD activity as low as 0.01 µU in samples.

L- Glutamate $\xrightarrow{\text{GAD/Cofactor}}$ CO_2 + GABA $\longrightarrow\longrightarrow$ NADPH + PicoProbe $\longrightarrow$ **Fluorescence (Ex/Em = 535/587 nm)**

II. Application:

- Measure GAD activity of pure enzyme
- Measure GAD activity in plant tissues (e.g. spinach, germinated barley seeds etc.)
- Measure GAD activity in animal tissues (e.g. mouse brain, mouse pancreas etc.)
- Mechanistic study of GAD

III. Sample Type:

- Plant tissues: e.g. Spinach and Germinated Barley Seeds
- Animal tissues: Brain and Pancreas

IV. Kit Contents:

Components	K2021-100	Cap Code	Part Number
GAD Assay Buffer	25 ml	WM	K2021-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K2021-100-2
GAD Substrate	400 µl	Green	K2021-100-3
GAD Cofactor	200 µl	White	K2021-100-4
GAD Converter	200 µl	Amber	K2021-100-5
GAD Developer	1 vial	Red	K2021-100-6
GAD Positive Control	1 vial	Orange	K2021-100-7
GAD Reconstitution Buffer	100 µl	Violet	K2021-100-8
NADPH Standard	1 vial	Yellow	K2021-100-9

V. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom
- Multi-well spectrophotometer (fluorescence plate reader)
- Multi-channel pipette
- 10 kDa Spin Column (BioVision Cat. # 1997)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Briefly spin the vials prior to opening. Read the entire protocol before performing the assay. Unless specified, 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 dH_2O 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.

VII. GAD Activity Assay Protocol:

1. Sample Preparation: Homogenize tissue (~25 mg) with 500 µl ice-cold GAD Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x *g*, 4°C for 20 min and collect the supernatant. Use 10 kDa spin column (BioVision Cat. # 1997) to remove small interfering molecules. Aliquot 100 µl Sample(s) in a clean centrifuge tube and add 400 µl of GAD Assay Buffer. Mix well. 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. 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. 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. Repeat 2 more times and save the Sample ultraconcentrate from the upper bucket (for testing the GAD activity). 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. 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. For GAD Positive Control **(PS)** well, add 2-20 µl of GAD Positive Control into the desired well(s). Adjust the volume of the PS, S and SBC wells to 50 µl/well with GAD Assay Buffer.

Notes:

a. 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.

b. Protein concentration in Samples can be determined using BCA Protein Assay Kit (BioVision Cat. # K818-1000).

2. NADPH Standard Curve: 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 dH_2O. 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. Adjust the volume to 50 µl/well with GAD Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed.

	Reaction Mix	***Background Control Mix**
GAD Assay Buffer	38 µl	42 µl
GAD Substrate	4 µl	--- µl
GAD Cofactor	2 µl	2 µl
GAD Converter	2 µl	2 µl
GAD Developer	2 µl	2 µl
PicoProbe™	2 µl	2 µl

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). Once a Sample is determined to have insignificant background it can be run without SBC.

4. Measurement: 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 (t_1 and t_2) 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).

5. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the NADPH Standard Curve. 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. 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 ($\Delta t = t_2 - t_1$).

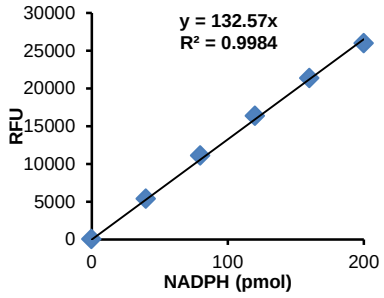
Sample GAD Activity = B/(Δt X V) x D = pmol/min/µl = µU/µl = mU/ml

Where:
B = The NADPH amount in the Sample well from Standard Curve (pmol)
Δt = 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.

(a)



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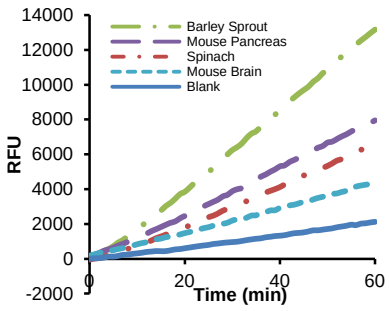

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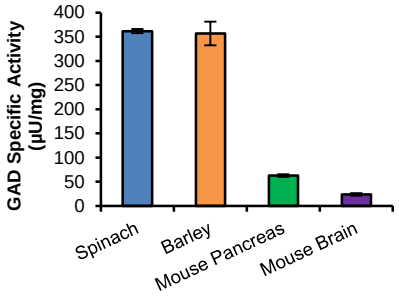

Figures: (a). NADPH Standard Curve. (b). Kinetic measurement of GAD Specific Activity in lysates prepared from Barley Sprout lysate (2 µg), Spinach lysate (2 µg), Mouse Pancreas lysate (12 µg), Mouse Brain lysate (12 µg) & GAD Positive Control. (c). GAD specific activity of Spinach, Barley Sprout, Mouse Pancreas & Mouse Brain lysates. Assay was performed following the kit protocol.

VIII. Related Products:

Glutamate Dehydrogenase Inhibitor Screening Kit (K185)
Glutamate Carboxypeptidase II Inhibitor Screening Kit (K440)
Glutamate Colorimetric Assay Kit (K629)
Glutamate Dehydrogenase Colorimetric Activity Assay Kit (K729)
PicoProbe Glutamate Assay Kit (K413)
Glutamine Colorimetric Activity Assay Kit (K556)
Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)
PicoProbe Glutamate Carboxylpeptidase II Activity Assay Kit (K738)

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