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CheKine™ Micro Glutamate Decarboxylase (GAD) Activity Assay Kit

Cat #: KTB3044 Size: 48 T/24 S 96 T/48 S

	Micro Glutamate Decarboxylase (GAD) Activity Assay Kit		
REF	**Cat #:** KTB3044	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal tissues, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glutamate Decarboxylase (GAD) is the rate-limiting enzyme that converts glutamate into the inhibitory neurotransmitter γ-aminobutyric acid (GABA), a potent inhibitory neurotransmitter in the central nervous system, It can reduce blood pressure, promote brain vitality, nourish nerve cells, maintain neurostability, promote growth hormone secretion, protect liver and benefit kidney, and so on. At present, it has been widely used in medicine and health food. CheKine™ Micro Glutamate Decarboxylase (GAD) Activity Assay Kit can be used to detect biological samples such as animal tissue, serum or plasma. In this kit, GAD catalysed glutamate to produce GABA, and the berthelot reaction was used to measure GABA content and thus GAD activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	30 mL	60 mL	4℃
Reagent Ⅰ	6 mL	12 mL	4℃, protected from light
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	2.5 mL	5 mL	4℃
Reagent Ⅳ	3 mL	6 mL	4℃
Reagent Ⅴ	6 mL	12 mL	4℃, protected from light
Reagent Ⅵ	6 mL	12 mL	4℃, protected from light
Reagent Ⅶ	12.5 mL	25 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 640 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tubes

- Water bath, freezing centrifuge
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer： Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃ protected from light.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃ protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent V: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃ protected from light.

Reagent VI: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃ protected from light.

Note: Reagent V is toxic, Reagent VI has a pungent odor, so it is recommended to experiment in a fume hood.

Reagent VII: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plasma ,or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 640 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Well (μL)	Control Well (μL)
Sample	100	0
Inactivated samples (Incubat at 95℃ for 10 min)	0	100
Reagent Ⅰ	100	100
Reagent Ⅱ	100	100
Mix well, and after 1 h of reaction in a water bath at 40℃, the reaction was terminated by a water bath at 95℃ for 10 min. The reaction solution was cooled to room temperature for use. The following operations were operated in a new 1.5 mL EP tube:		
Reaction Solution	40	40
Reagent Ⅲ	10	10
Reagent Ⅳ	50	50
Reagent Ⅴ	100	100
The mixture was mixed and allowed to stand at room temperature for 5 min.		

Reagent Ⅵ	100	100
The mixture was mixed and allowed to stand at room temperature for 5 min.		
Reagent Ⅶ	200	200

3.Mix thoroughly, add 200 μL to 96-well plate or microglass cuvette, and measure the absorbance value at 640 nm. The Control Well is recorded as $A_{Control}$, and the test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$.

Note: Each test well needs to be equipped with a control well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

Under standard conditions, the regression equation is y=0.0682x-0.0432, R^2=0.999. X is the standard concentration (μmol/mL), y is the absorbance value

2. Calculation of GAD activity:

(1) Calculated by protein concentration

Active unit definition: The catalytic production of 1 nmol GABA per minute per milligram of protein was defined as one unit of enzyme activity.

GAD(U/mg prot)=$(\Delta A_{Test}+0.0432)\div0.0682\times V_{Total}\div(V_{Sample}\times Cpr)\div T\times1,000$**$=733\times(\Delta A_{Test}+0.0432)\div Cpr$**

(2) Calculated by sample fresh weight

Active unit definition:The catalytic production of 1 nmol GABA per minute per gram of sample was defined as one unit of enzyme activity.

GAD(U/g fresh weight)=$(\Delta A+0.0432)\div0.0682\times V_{Total}\div(W\times Vsample\div V_{Sample\ total})\div T\times1,000$**$=733\times(\Delta A_{Test}+0.0432)\div W$**

(3) Calculated by volume of liquid sample

Active unit definition:The catalytic production of 1 nmol GABA per minute per milliliter of liquid is one unit of enzyme activity.

GAD(U/mL)=$(\Delta A+0.0432)\div0.0682\times V_{Total}\div(V_{Sample}\div V_{Sample\ total})\div T\times1,000$**$=733\times(\Delta A_{Test}+0.0432)$**

V_{Total}: total reaction volume, 0.3 mL; $V_{Sample:}$ sample volume added, 0.1 mL; $V_{Sample\ tota}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 1,000: conversion factor, 1 μmol=1,000 nmol.

Typical Data

The following data are for reference only, and the experimenter is required to test the samples according to their own experiments.

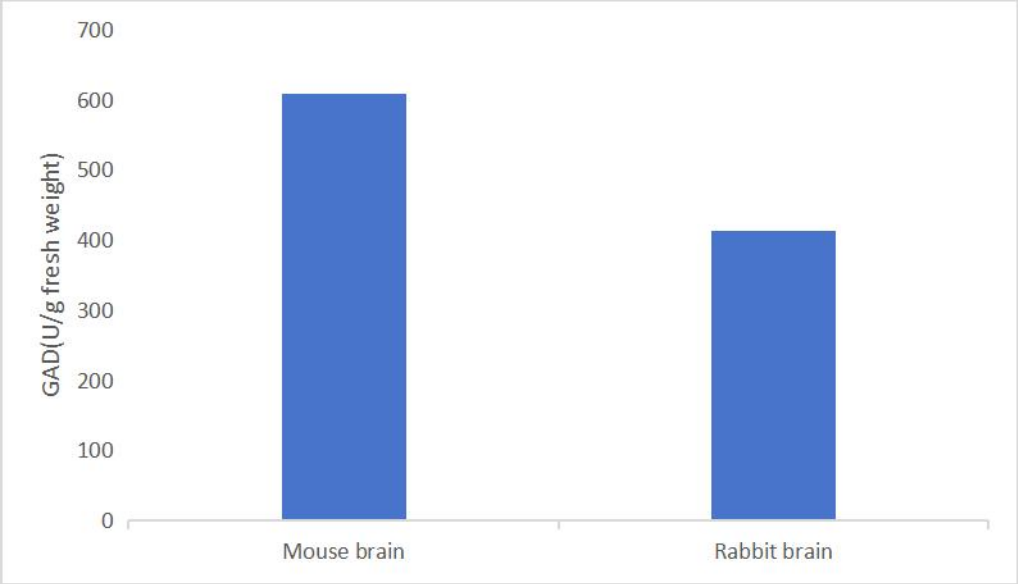

Figure 1. Determination GAD activity in mouse and rabbit brain by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1125	CheKine™ Micro Pyruvate Decarboxylase (PDC) Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase (GDH) Assay Kit
KTB1110	CheKine™ Lactate Dehydrogenase (LDH) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.