Gamma-Aminobutyric Acid Assay Kit

Catalog No.: abx295111

Size: 96 tests

Detection Range: 0.06 µmol/ml – 10.0 µmol/ml

Sensitivity: 0.06 µmol/ml

Storage: Store all components at 4°C in the dark.

Application: For detection and quantification of Gamma-Aminobutyric Acid in tissue homogenates.

Introduction

Abbexa's Gamma-Aminobutyric Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Gamma-Aminobutyric Acid (GABA) concentration. GABA present in samples reacts with phenol and sodium hypochlorite to produce a compound that has an absorption maximum at 640 nm. The intensity of blue-green color is proportional to the concentration of GABA, which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 60 ml
3. Buffer Solution: 6 ml
4. Chromogenic Reagent A: 4.8 ml
5. Chromogenic Reagent B: 7.2 ml
6. Diluent Buffer: 24 ml
7. GABA Standard (10 µmol/ml): 2 × 1.6 ml
8. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (640 nm)
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. Vials/tubes
5. Microcentrifuge tubes
6. Centrifuge
7. Mechanical homogenizer
8. Incubator or water bath (95°C)
9. Vortex mixer
10. Ice

Protocol

A. Preparation of samples and reagents

1. Samples

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue homogenates:** Weigh at least 0.05 g of tissue sample and wash in cold PBS (0.01 M, pH 7.4). Per 0.05 g add 450 µl of Extraction Solution and homogenize manually using a mechanical homogenizer at 4°C. Transfer to a new tube, and mark the level of liquid on the tube. Seal the tube with plastic wrap, leaving a small hole for ventilation. Incubate at 95°C for 2 hours. Add Extraction Solution to restore the sample solution to the original volume marked on the tube, and mix fully. Centrifuge at 8000 × g for 10 minutes. Transfer the supernatant to a new tube and analyze immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% *Epipremnum aureum* tissue homogenate	1
10% Green pepper tissue homogenate	1
10% Yam tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.

2. Reagents

1. Bring all reagents to room temperature prior to use.
2. **Standards:** Label 8 tubes with 10 µmol/ml, 9 µmol/ml, 7 µmol/ml, 5 µmol/ml, 4 µmol/ml, 2 µmol/ml, 1 µmol/ml, and 0 µmol/ml. Add 200 µl, 180 µl, 140 µl, 100 µl, 80 µl, 40 µl, 20 µl, and 0 µl of Standard (10 µmol/ml) to the 10 µmol/ml, 9 µmol/ml, 7 µmol/ml, 5 µmol/ml, 4 µmol/ml, 2 µmol/ml, 1 µmol/ml, and 0 µmol/ml tubes respectively, followed by 0 µl, 20 µl, 60 µl, 100 µl, 120 µl, 160 µl, 180 µl and 200 µl of Extraction Solution, to prepare Standard Dilutions with concentrations 10 µmol/ml, 9 µmol/ml, 7 µmol/ml, 5 µmol/ml, 4 µmol/ml, 2 µmol/ml, 1 µmol/ml, and 0 µmol/ml. These volumes are summarized in the following table:

Standard Dilution (µmol/ml)	10	9	7	5	4	2	1	0
10 µmol/ml Standard (µl)	200	180	140	100	80	40	20	0
Extraction Solution (µl)	0	20	60	100	120	160	180	200

For the blank, or 0 µmol/ml standard, use pure Extraction Solution. The volume of each standard will be 200 µl.

B. Assay Procedure.

1. Set standard and sample tubes. *It is strongly recommended to prepare all tubes in duplicate.*
2. Add 30 µl of each standard dilution to the corresponding standard tubes.
3. Add 30 µl of each sample to the sample tubes.
4. Add 50 µl of Buffer Solution and 40 µl of Chromogenic Reagent A to all tubes.
5. Mix fully with a vortex mixer and allow to stand for 5 minutes at room temperature.
6. Add 60 µl of Chromogenic Reagent B to all tubes. Mix fully with a vortex mixer.
7. Incubate at 95°C for 10 minutes, then cool in an ice bath.
8. Add 200 µl of Diluent Buffer to all tubes and mix fully.
9. Set standard and sample wells on the microplate and record their positions.
10. Take 200 µl from each tube to the respective microplate wells.
11. Tap the plate gently to mix. Read and record the absorbance at 640 nm with a microplate reader. **Absorbance must be read within 10 minutes of adding Diluent Buffer.**

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. The GABA concentration in each sample well can be derived with the following formula:

$$\text{GABA}\ (\mu\text{mol/g}) = \frac{\Delta\text{A} - \text{b}}{\text{a}} \times \frac{\text{V} \times \text{f}}{\text{w}}$$

where:

ΔA	OD value of the sample ($\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}$)
a	Gradient of the standard curve ($\text{y} = \mathbf{a}\text{x} + \text{b}.$)
b	Y-intercept of the standard curve ($\text{y} = \text{ax} + \mathbf{b}$)
f	Dilution factor of the sample before carrying out the assay
V	Volume of Extraction Solution added (ml)
w	The wet weight of tissue (g)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.