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 yellow 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. Reaction Buffer: 6 ml
4. Detection Reagent A: 4.8 ml
5. Detection 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. Pipette and pipette tips
3. Vials/tubes
4. Incubator or water bath (95°C)
5. Vortex mixer
6. Ice

Protocol

A. Preparation of 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:** Homogenize 0.05 g of sample in 450 µl of Extraction Solution. Transfer to a new tube, and mark the level of liquid on the tube. Seal the tube, leaving a small hole for ventilation. Incubate at 95°C for 2 hours. Add Extraction 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.

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

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

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. **Standard curve preparation:** Label 8 tubes with 0, 1.0, 2.0, 4.0, 5.0, 7.0, 9.0, and 1.0 µmol/ml. Prepare the standard tubes according to the following table.

Volume of 10 µmol/ml Standard (µl)	Volume of Extraction Solution (µl)	Standard concentration (µmol/ml)
0	200	0
20	180	1.0
40	160	2.0
80	120	4.0
100	100	5.0
140	60	7.0
180	20	9.0
200	0	10.0

2. Set standard and sample tubes.
3. Add 30 µl of each standard to the respective standard tubes.
4. Add 30 µl of each sample to the sample tubes.
5. Add 50 µl of Reaction Buffer and 40 µl of Detection Reagent A to all tubes.
6. Mix fully with a vortex mixer and allow to stand for 5 minutes at room temperature.
7. Add 60 µl of Detection Reagent B to all tubes. Mix fully with a vortex mixer.
8. Incubate at 95°C for 10 minutes. Cool in an ice bath.
9. Add 200 µl of Diluent Buffer to all tubes and mix fully.
10. Set standard and sample wells on the microplate and record their positions.
11. Take 200 µl from each tube to the respective microplate wells.
12. 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

The standard curve can be plotted as the absolute OD_{640} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The GABA concentration of the samples can be interpolated from the standard curve.

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

where:

ΔA	OD value of the sample ($\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
V	Volume of Extraction Solution added (ml)
m	The wet weight of tissue (g)