Glutaminase Assay Kit (BA0218)

Product Datasheet | SKU BA0218

Description

Glutaminase (EC 3.5.1.2) is a key enzyme in the metabolism of glutamine. Glutamine is an important amino acid in the metabolism of cancer cells, and increased glutaminolysis has been linked to cancer. This kit provides a convenient fluorimetric method to measure glutaminase activity in biological samples. In the assay, o-phthalaldehyde reacts with liberated ammonia, and the increase in fluorescence at lambda ex/em = 415/475 nm is directly proportional to enzyme activity.

Specifications

Detection Method	Fluorimetric, kinetic; read at lambda ex/em = 415/475 nm
Detection Range	0.066-50 U/L glutaminase
Sample Type	Serum, plasma, urine, biological samples
Species Reactivity	All
Assay Time	Within 50 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store all components at -20 degrees C upon receipt.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer	5 mL	-20 degrees C
20 mM Substrate	500 uL	-20 degrees C
20 mM Standard	50 uL	-20 degrees C
Detection Reagent	5 mL	-20 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0218	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. This assay is based on a kinetic reaction, so addition of the Working Reagent to samples should be quick and mixing brief but thorough; use of a multi-channel pipettor is recommended and the assay can be run at room temperature.
2. Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening; the Working Reagent should be prepared fresh for each assay run.
3. Prepare enzyme in an enzyme buffer such as 20 mM potassium phosphate, pH 7.4; the protocol is optimised for glutaminase from Bacillus amyloliquefaciens, and for other enzymes experimentally determine the optimal amount per well.
4. Dilute serum and plasma samples at least 1:10, and dilute urine 1:50 in water prior to the assay run.
5. Prepare a 1 mM Standard Premix by combining 10 microlitres of 20 mM Standard with 190 microlitres of distilled water, then prepare all standards according to the standard preparation table and transfer 10 microlitres of each Standard to separate wells of a black 96-well plate.

6. Transfer 10 microlitres of each sample to separate wells of the plate.
7. Prepare enough Working Reagent for all assay wells by mixing 5 microlitres of 20 mM Substrate and 45 microlitres of Assay Buffer for each well.
8. Initiate the reaction by adding 40 microlitres of Working Reagent to all assay wells, tap the plate to mix and incubate for 30 minutes at room temperature.
9. Add 50 microlitres of Detection Reagent to all wells, tap the plate to mix and read for 20 minutes, measuring fluorescence intensity at lambda ex/em = 415/475 nm.

Standard Preparation

No.	Premix + dH2O	Total Volume	Std (uM)
1	100 uL + 0 uL	100 uL	1000
2	60 uL + 40 uL	100 uL	600
3	30 uL + 70 uL	100 uL	300
4	0 uL + 100 uL	100 uL	0

Calculation

Subtract the blank value (Standard #4) from the standard values and plot delta-F against the standard concentrations. Determine the slope (uM-1) and calculate glutaminase activity as: Glutaminase Activity = [(F_Sample - F_Blank) / (Slope (uM-1) x t)] x n (U/L), where F_Sample and F_Blank are the measured fluorescence values of the sample and blank, t is the reaction time (30 min) and n is the sample dilution factor. Unit definition: 1 Unit (U) of glutaminase will catalyse the conversion of 1 micromole of L-glutamine per minute at room temperature and pH 7.4. Note: if sample glutaminase activity exceeds 50 U/L, dilute samples in enzyme buffer and repeat the assay.

Notes

- This assay is based on a kinetic reaction; addition of the Working Reagent should be quick and mixing brief but thorough.
- The Working Reagent should be prepared fresh for each assay run.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.