QuantiFluo™ Glutaminase Assay Kit (DGLN-100)

Quantitative Fluorimetric Assay for Glutaminase Activity

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. BioAssay Systems' DGLN-100 Kit provides a convenient fluorimetric method to measure glutaminase activity in biological samples. In this assay, o-phthalaldehyde reacts with liberated ammonia where the increase in fluorescence at $\lambda_{ex/em}$ = 415/475 nm is directly proportional to enzyme activity.

KEY FEATURES

Fast and Safe. Assay can be completed within 50 minutes. Non-radioactive assay.

Sensitive and accurate. Linear detection range is 0.66-500 U/L glutaminase in a 96-well plate assay.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of glutaminase activity in biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 5 mL **20 mM Standard:** 50 µL
20 mM Substrate: 500 µL **Detection Reagent:** 5 mL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent (*WR*) to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. The assay can be run at room temperature.

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The *WR* should be prepared fresh for each assay run.

Enzyme Preparation: Enzyme should be prepared in an enzyme buffer, e.g. 20 mM Potassium Phosphate, pH 7.4. The following protocol is optimized for glutaminase from *Bacillus amyloliquefaciens*. If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Sample Preparation:

Serum and *Plasma* samples must be diluted at least 1:10.

Urine must be diluted 1:50 in water prior to the assay run.

Standard Preparation: Prepare a 2 mM Standard Premix by combining 20 µL 20 mM Standard and 180 µL Assay Buffer. Prepare all standards according to table below. Transfer 10 µL of Standard to each well plus 40 µL of Assay Buffer.

No.	2 mM Std Premix + Assay Buffer	Total Volume (µL)	Std (mM)
1	100 µL + 0 µL	100 µL	2 mM
2	50 µL + 50 µL	100 µL	1 mM
3	25 µL + 75 µL	100 µL	0.5 mM
4	0 µL + 100 µL	100 µL	0 mM

Reaction Preparation:

1. Transfer 10 µL of each sample to separate wells of the plate.
2. Prepare enough *WR* for all sample wells by mixing 5 µL of 20 mM Substrate and 45 µL of Assay Buffer for each well.
3. Initiate the reaction by addition of 40 µL of *WR* to all sample wells. Incubate the reaction for 30 minutes at RT.
4. Add 50 µL *Detection Reagent* to all wells. Tap plate to mix and read for 20 min. Measure fluorescence intensity at $\lambda_{ex/em}$ = 415/475 nm.

CALCULATION

Subtract the blank value (Standard #4) from the standard values and plot ΔF against the standard concentrations. Determine the slope (mM^{-1}) and calculate the glutaminase activity in each Sample as follows,

$$\text{Glutaminase Activity} = \frac{(F_{Sample} - F_{Blank})}{\text{Slope } (mM^{-1})} \times \frac{\text{Reaction Vol}}{t\,(\text{min}) \times \text{Enzyme Vol } (\mu L)} \times n \text{ (U/L)}$$

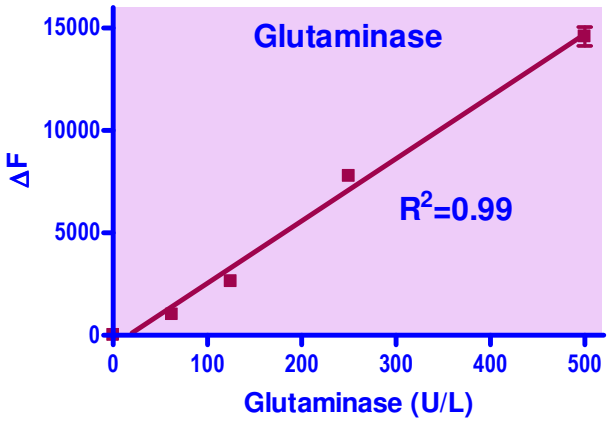
Where F_{Sample} and F_{Blank} are the measured fluorescence values of the sample and blank, t is the reaction time (30 min), Reaction Vol and Enzyme Vol are 50 µL and 10 µL, respectively, and n is the sample dilution factor.

Unit definition: 1 Unit (U) of glutaminase will catalyze the conversion of 1 micromole L-glutamine per min at room temperature and pH 7.4.

Note: If sample glutaminase activity exceeds 500 U/L, dilute samples in enzyme buffer and repeat the assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and a plate reader.



Varying amounts of Glutaminase were assayed in the presence of 2 mM Glutamine for 30 minutes at RT.

LITERATURE

1. Milano, S. K., et al (2022). New insights into the molecular mechanisms of glutaminase C inhibitors in cancer cells using serial room temperature crystallography. Journal of Biological Chemistry, 298(2), 101535.

2. Sugawara, K, Oyama, F. (1981). Fluorgenic reaction and specific microdetermination of ammonia. J. Biochem. 89, 771–774.