Glutamine Assay Kit

Catalog Number KA1627

100 assays

Version: 05

Intended for research use only

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Table of Contents

Introduction

Intended Use

Application

- ✓ Direct Assays: glutamine in serum, plasma, urine, tissue extracts and cell culture samples.
- ✓ Drug Discovery/Pharmacology: effects of drugs on glutamine metabolism.

Features

- ✓ Sensitive and accurate: Use 20 µL sample. Linear detection range 0.023 - 2 mM glutamine in 96-well plate assay.
- ✓ Convenient: The procedure involves adding a single working reagent, incubation for 40 min at room temperature, adding a stop reagent and reading the optical density. No 37℃ heater is needed.
- ✓ High-throughput: Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Background

Glutamine is an amino acid synthesized in the muscle that plays major roles in protein synthesis, acid-base balance, anabolic processes and is utilized for cellular energy and as a carbon source. It is used in treatment of injury, trauma, burns, and also as a supplement for muscle growth and post-surgery healing.
Simple, direct and automation-ready procedures for measuring glutamine concentration are very desirable. Glutamine Assay Kit is based on hydrolysis of glutamine to glutamate and colorimetric determination of the product. The intensity of the product color, measured at 565 nm, is proportional to the glutamine concentration in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer	15 mL
NAD Solution	1 mL
Enzyme A	120 µL
MTT Solution	1.5 mL x 2
Enzyme B	220 µL
Stop Reagent	25 mL
Standard (100 mM Glutamine)	400 µL

Materials Required but Not Supplied

- ✓ Pipeting (multi-channel) devices
- ✓ Clear flat-bottom 96-well plates (e.g. Corning Costar)
- ✓ Plate reader.

Storage Instruction

Store all reagents at -20℃. Shelf life of 6 months after receipt.

Precautions for Use

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Assay Procedure

Note:

1. *This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.*
2. *The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).*

1. Standard Curve. Prepare 2.0 mM glutamine Premix by mixing 5 µL 100 mM Standard and 245 µL distilled water. Dilute standard as follows. Transfer 20 µL standards into wells of a clear flat-bottom 96-well plate.

No	Premix + H_2O	Vol (µL)	Glutamine (mM)
1	100 µL + 0 µL	100	2.0
2	60 µL + 40 µL	100	1.2
3	30 µL + 70 µL	100	0.6
4	0 µL + 100 µL	100	0.0

   Samples: add 20 µL sample per well in separate wells.

   IMPORTANT: if a sample is known to contain glutamate, a sample blank control is required. In this case, transfer an additional 20 µL sample into a separate well.

2. Reaction. Spin the enzyme and reagent tubes briefly before pipetting. Fresh reconstitution is recommended.

   For each standard and sample well, prepare Working Reagent by mixing 65 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 2.5 µL NAD and 14 µL MTT.

   Where a sample blank is required, prepare a Blank Working Reagent by mixing 65 µL Assay Buffer, 1 µL Enzyme B, 2.5 µL NAD and 14 µL MTT (i.e. No Enzyme A).

   Add 80 µL Working Reagent per well to standards and sample wells.

   Where appropriate, add 80 µL Blank Working Reagent to the Sample Blank wells.

   Tap plate to mix briefly and thoroughly.

3. Incubate 40 min at room temperature. Add 100 µL Stop Reagent to each well. Read OD at 565 nm (520-600 nm).

Data Analysis

Calculation of Results

Subtract water (#4) blank OD from OD values for the standards. Plot ΔOD against standard concentrations. Determine the slope and calculate sample glutamine concentration,

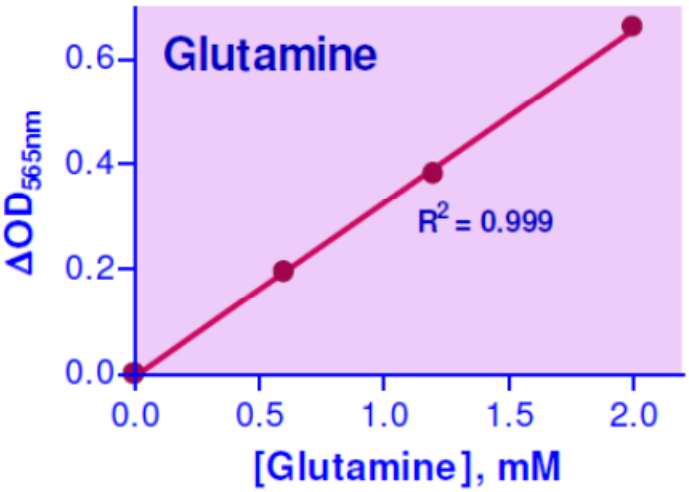
$$[\text{Glutamine}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}\,(mM^{-1})} \times n \quad (mM)$$

where OD_{SAMPLE} and OD_{BLANK} are the OD values of the sample and water (if sample does not contain glutamate) or sample blank (if sample contains glutamate).

Note: if the calculated glutamine concentration is higher than 2 mM, dilute sample in distilled water and repeat the assay. Multiply the results by the dilution factor n.

Conversions: 1 mM glutamine = 14.6 mg/dL or 146 ppm.

Standard Curve in 96-well plate assay



Resources

References

1. Behjousiar A et al (2012). In situ monitoring of intracellular glucose and glutamine in CHO cell culture. PLoS One 7(4):e34512.
2. Lin TC et al (2012). Autophagy: Resetting glutamine-dependent metabolism and oxygen consumption. Autophagy 8(10):1477- 1493.
3. Tang N et al (2012). Stable overexpression of arginase I and ornithine transcarbamylase in HepG2 cells improves its ammonia detoxification. J Cell Biochem. 113(2):518-527.