Technical Bulletin

Glutamine Assay Kit

Catalog Number MAK438

Product Description

Glutamine is an amino acid synthesized in the muscle that plays major roles in protein synthesis, acid-base balance, and anabolic processes. Glutamine is utilized for cellular energy and as a carbon source. It is used in the 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. The Glutamine Assay Kit is based on the hydrolysis of glutamine to glutamate and the colorimetric determination of the product. The intensity of the product color, measured at 565 nm, is proportional to the glutamine concentration in the sample. The linear detection range for the assay method is 0.02 to 2 mM glutamine.

The kit is suitable for the quantitative determination of glutamine and evaluation of drug effects on glutamine metabolism in serum, plasma, urine, cultured cells, and tissue extracts.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer — 15 mL
  Catalog Number MAK438A
- NAD Solution — 1 mL
  Catalog Number MAK438B
- Enzyme A — 120 µL
  Catalog Number MAK438C
- MTT Solution — 2 x 1.5 mL
  Catalog Number MAK438D
- Enzyme B — 220 µL
  Catalog Number MAK438E
- Stop Reagent — 25 mL
  Catalog Number MAK438F
- Standard (100 mM Glutamine) — 400 µL
  Catalog Number MAK438G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Note: The following substances interfere with the assay reaction and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and TWEEN® 20 (>1%).

1. All samples should be clear and free of any turbidity or particles. Add 20 µL of Sample to separate wells of a clear 96-well plate.
2. If a Sample is known to contain glutamate, a Sample Blank is required. In this case, transfer an additional 20 µL of each Sample into a separate well of the plate for use as the Sample Blank.

Standard Curve Preparation

1. Prepare a 2.0 mM Glutamine Standard by mixing 5 µL of the 100 mM Glutamine Standard with 245 µL of purified water.
2. Prepare Glutamine standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Glutamine Standards

Well	2.0 mM Glutamine	Purified Water	Glutamine (mM)
1	100 µL	-	2.0
2	60 µL	40 µL	1.2
3	30 µL	70 µL	0.6
4	-	100µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of the plate.

Working Reagents

Note: 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 a multichannel pipettor is recommended.

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 2.
   a. For each Standard and Sample well, prepare 83.5 µL of Working Reagent.
   b. Where a Sample Blank is required, prepare 82.5 µL of Blank Working Reagent for each Sample Blank well.

Table 2.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	65 µL	65 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL
NAD Solution	2.5 µL	2.5 µL
MTT Solution	14 µL	14 µL

Measurement

1. **Quickly** add 80 µL of Working Reagent to each Sample and Standard well. Tap plate to mix.
2. If applicable, quickly add 80 µL of Blank Working Reagent to each Sample Blank well. Tap plate to mix.
3. Incubate the plate for 40 minutes at room temperature.
4. Add 100 µL of Stop Reagent to each well.
5. Read optical density at 565 nm (OD_{565}).

Results

1. Subtract the 0 Standard OD_{565} reading from all Standard OD_{565} readings.
2. Plot the corrected Standard OD_{565} readings against Standard concentrations. Determine the slope of the Standard curve.
3. Calculate the Sample glutamine concentration:

$$\text{Glutamine (mM)} = \frac{OD_S - OD_B}{\text{Slope } (mM^{-1})} \times DF$$

where

OD_S = OD_{565} reading of Sample

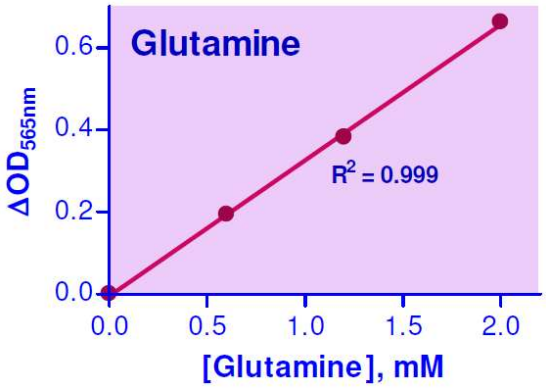
OD_B = OD_{565} reading of Sample Blank

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated glutamine concentration is higher than the 2.0 mM Standard, dilute the Sample in purified water and repeat the assay.

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

Figure 1.
Typical Glutamine Standard Curve



References

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2. Tran, T.Q., et al., α-Ketoglutarate attenuates Wnt signaling and drives differentiation in colorectal cancer. *Nat. Cancer*, **1(3)**, 345-358 (2020).
3. Ma, H., et al (2018). Inhibition of SLC1A 5 sensitizes colorectal cancer to cetuximab. *Int. J. Cancer*, **142(12)**, 2578-2588 (2018).

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