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ProductInformation

GLUTAMINE ASSAY KIT

Stock No. **GLN-2**

Store at –20 °C

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description

The Glutamine Assay Kit is a quantitative, colorimetric assay specifically designed for use in cell culture. The assay is based on the reductive deamination of L-glutamine by a proprietary enzyme. Quantitation is accomplished by linking a dye directly to the reductive reaction. The reaction is specific for L-glutamine and does not cross-react with other amino acids or ammonia. A variable amount of inhibition/enhancement is observed with different media formulations therefore, an internal standard of L-glutamine should be included in the assay. The protocol for this step and the calculations are described below. The colored reaction product is read at a wavelength of 550 nm using a standard spectrophotometer.

REAGENT
For Research Use Only.
Not for Use in Diagnostic Procedures.

Kit Components

Catalog No.	Item	Quantity
G7531	Glutamine Assay: Reaction Buffer	3.0 ml
G7406	Glutamine Assay: Enzyme Preparation	7.5 ml
G7156	Glutamine Assay: Color Reagent	5.0 ml
G7656	Glutamine Assay: Standard (60 mM L-glutamine)	5.0 ml
G7281	Glutamine Assay: Diluent Buffer, 10x	10.0 ml

WARNING: The color reagent (Product Code G 7156) in this kit contains hydrochloric acid. Please exercise due caution.

Materials Required but Not Provided

Spectrophotometer
Vortex Mixer
Micropipetors
37 °C Waterbath
Test Tubes, 10 x 75 mm or equivalent

Product Storage

Store at –20 °C or lower.
The Reaction Buffer, Product Code G 7531 will spontaneously degrade and generate increased background levels if stored at temperatures higher than –20 °C for more than 1 week.
The color reagent contains 2N HCl and should be handled with caution.
Some reagents may not freeze due to their composition.

Procedure

The glutamine assay was developed for use in cell culture. For best results the assay should be performed in duplicate with a standard curve range extending from 0.25 mM to 6.0 mM L-glutamine. The limit of sensitivity can be increased beyond 0.25 mM by extending the incubation time. Similarly, the sensitivity of the assay can be reduced by shortening the incubation period.

NOTE: Precipitate may be visible in the Enzyme Preparation Product Code G 7406. If desired, the solution may be filtered or centrifuged at 2000 RPM for two minutes to remove the precipitate. Product performance will not be affected.

Preparation of Standard Curve

1. Prepare 1x Diluent Buffer Product Code G 7281 by adding the contents of the vial to 90 ml of deionized water. This reagent is stable for at least 2 months when stored at 2-8 °C. Additional 1x Diluent buffer may be produced by preparing a 0.1 M solution of Tris-HCl, pH 8.0.
2. The recommended standard curve contains the following standards:0 mM, 0.25 mM, 0.50 mM, 1.0 mM, 2.0 mM, 3.0 mM and 6.0 mM. The standards should be prepared in duplicate. The standard curve may be altered to meet the individual user's requirements.

3. To prepare the 6.0 mM L-glutamine standard, remove 0.5 ml from the standard vial and add it to 4.5 ml 1x Diluent Buffer. Mix.
4. To prepare the 3.0 mM standard add 1.0 ml of the 6.0 mM standard to 1.0 ml of Diluent buffer. Mix.
5. To prepare the 2.0 mM standard add 2.0 ml of the 6.0 mM standard to 4.0 ml of Diluent buffer. Mix.
6. To prepare the 1.0 mM standard add 2.0 ml of the 2.0 mM standard to 2.0 ml of Diluent buffer. Mix.
7. To prepare the 0.5 mM standard add 2.0 ml of the 1.0 mM standard to 2.0 m of Diluent buffer. Mix.
8. To prepare the 0.25 mM standard add 2.0 ml of the 0.5 mM standard to 2.0 ml of Diluent buffer. Mix.

Preparation of Internal Standard

The internal standard is included to detect any inhibition or enhancement of the reaction caused by the components of the sample medium. The internal standard is prepared by adding a 10 µl aliquot of the undiluted Standard to a tube containing a 300 µl aliquot of the sample medium to be tested.

1. Prepare a sufficient number of test tubes to run internal standard in duplicate. An internal standard should be prepared for each type of medium assayed.
2. Add 50 µl of reaction buffer to each tube.
3. Add 300 µl of sample to each tube.
4. Add 10 µl of undiluted L-glutamine Standard and 490 µl 1x Diluent buffer to each of the internal standard tubes. Note: The volume of Standard will yield an internal standard concentration of 2 mM. The individual user may select different or additional concentrations for the internal standard.

Process Internal Standard with Samples and Standards starting with Step 5 of Assay Procedure below.

Assay Procedure

1. Prepare a sufficient number of test tubes to run assay in duplicate including one pair of tubes for each internal standard (see above). An internal standard should be prepared for each type of medium assayed.
2. Add 50 µl of reaction buffer to each tube.
3. Add 300 µl of sample or standard to each tube.

Note: Make sure that the sample is run both with (internal standard) and without (sample) the L-glutamine spike as this information is needed to calculate the recovery value.

4. Add 500 µl 1x Diluent buffer to each of the remaining sample and standard tubes.
5. Add 150 µl of enzyme preparation to each tube and mix thoroughly.
6. Incubate tubes for 1 hour in a 37 °C waterbath.
7. Add 100 µl of color reagent to each tube. Mix thoroughly until color is uniformly distributed. Let stand at room temperature for 5 minutes.
8. Transfer contents of tubes to 1 ml cuvets if desired and read at 550 nm using a spectrophotometer. Note: Reading should be made within 30 minutes following addition of color reagent.

Calculations

Standard curve: To calculate the quantity of L-glutamine in the samples perform a linear regression analysis of the standard curve. The slope of the regression line may be used to calculate the uncorrected L-glutamine concentration of the samples.

Internal standard: The recovery of the internal standard is used to correct the data obtained in the assay for deviations caused by the interaction of media components with the test. The following formula is used to calculate the recovery value:

$$\text{Recovery} = D = (A-B)/C$$

Where:
[L-glutamine] = mM concentration of L-glutamine
A = [L-glutamine] in sample with internal standard (spiked sample)
B = [L-glutamine] in sample without internal standard (unspiked sample)
C = [L-glutamine] added as internal standard (2 mM is the concentration when 10 µl of standard is used)
D = Recovery value

Corrected sample values: The concentration of L-glutamine in the sample tubes is corrected for any inhibition/enhancement using the recovery value of the internal standard calculated above according to the following formula:

$$[\text{L-glutamine}]\ \text{Corrected} = E/D$$

Where:
D = Recovery value Previously calculated.
E = [L-glutamine] Uncorrected in unspiked sample. Calculated from the standard curve.

Possible Sources of Error

1. Failure to include an internal standard for the specific medium being tested.
2. Using expired material. The 1x Diluent buffer should not be stored for more than 2 months after preparation.
3. Samples were not read within 30 minutes following addition of color reagent.

References

1. Kemp, C.W., and Shiloach, J., 1993
A simple colorimetric assay for the determination of L-glutamine in samples of tissue culture medium. American Biotechnology Laboratory, **11,** 9:12 (1993).

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