

Bioprocess Series

SIMULTANEOUS MEASUREMENT OF GLUTAMINE AND GLUTAMATE

Application Note 101LS
YSI Life Sciences



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INTRODUCTION

Glutamine and Glutamate concentrations in complex matrices can be measured using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glutamine and glutamate measurements. Measurements are virtually unaffected by color, turbidity, density, pH or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glutamine and a glutamate membrane, simultaneous measurement of both analytes is possible. Because glutamate interferes with the glutamine analysis, it is necessary to follow this protocol when analyzing for glutamine in the presence of glutamate.

When a sample is injected into the sample chamber, the glutamine diffuses to the glutamine membrane, which contains glutaminase and glutamate oxidase. The glutamine is deaminated to glutamate and ammonia. In the presence of glutamate oxidase, glutamate is oxidized to hydrogen peroxide, α -ketoglutarate, and ammonia. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration and hence to the glutamate concentration.

The glutamate in the sample is also oxidized at the glutamate and glutamine membranes, producing hydrogen peroxide, α -ketoglutarate, and ammonia. The 2900 Series calculates the glutamine concentration by subtracting the glutamate response from the glutamine response. In this way both glutamate and glutamine can be measured simultaneously.

MATERIAL AND SET UP

- A. YSI 2900 Series Biochemistry Analyzer- equipped with a 2735 Glutamine Membrane, a 2754 Glutamate Membrane and 2357 Buffer.
- B. Glutamine (5.00 mmol/L, 8.00 mmol/L) and Glutamate (5.00 mmol/L, 10.0 mmol/L) standard solutions. Refer to Method C of this note.
- C. Connect the 2900 Series instrument to a suitable power source.
- D. Perform the instrument and membrane daily checks described in the Operations Manual.
- E. Volumetric glassware (Class A recommended).
- F. The following instrument set up is recommended:
Sample size: 20 μ L*

Probe A Parameters

Chemistry	Glutamate
Unit	mmol /L
Calibrator	5.00 mmol /L
End Point	30 Sec

Probe B Parameters

Chemistry	Glutamine
Unit	mmol /L
Calibrator	5.00 mmol /L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	1 Sam
Cal Shift	2%

* can be changed to improve linearity (see manual).

METHOD

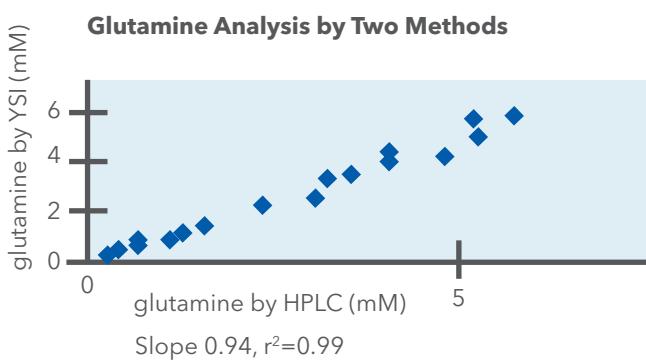
- A. The combined concentration of glutamine and glutamate should not exceed 10.0 mmol/L. If sum of the values reported exceeds this, further dilution of the sample is required.
- B. To make the 5 mmol/L standard, transfer one container of glutamine powder into a bottle of 2736 buffer solution (250 ml). For the 8 mmol/L standard, transfer one container of the glutamine powder into a bottle of 2737 buffer solution (156.25 ml).
- C. Calibrate the 2900 series with 5.00 mmol/L glutamate and 5.00 mmol/L glutamine calibration standards.
- D. Check the linearity of the membranes at least once a day by injection of glutamate (10 mmol/L) and *continued*

glutamine (8 mmol/L) linearity check solutions. Refer to the Operations Manual for specifications.

- E. Assay the sample by aspiration into the 2900 Series instrument. The linear range of the system is 0.2 to 8.0 mmol/L glutamine, and 0.1 to 10mmol/L glutamate, with an absolute error of approximately 0.3 mmol/L. If the value exceeds this further dilution is required.
- F. Calibrate frequently as described in the Operations Manual.

RESULTS

The graph below is the result of testing the YSI 2900 Series instrument vs. an HPLC for glutamine concentration, in cell culture media. (Data courtesy of a well established Biotechnology company.)



ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2735	Glutamine Membrane Kit
2754	Glutamate Oxidase Membrane Kit
2736	Glutamine Standard Solution (5.00mmol/L)
2737	Glutamine Standard Solution (8.00 mmol/L)
2755	L-Glutamate Standard Solution (5.00 mmol/L)
2756	L-Glutamate Standard Solution (10.0 mmol/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation)

NOTES: Glutamine standards can be stored at 4°C for up to one month. At room temperature glutamine is destroyed at an accelerated rate and standards will only retain accurate concentrations for a few days (see Sigma Chemical Company catalogue). Glutamate standards can be stored at room temperature for one month after opening without compromising the concentration of glutamate.

Observations of instruments running with glutamine and glutamate mixed samples have shown that after several months the sample chamber of the 2900 Series instrument can become infected with microbial contamination. This leads to a conversion of the glutamine to glutamate and thus back-ground readings to glutamine at the glutamate probe. This can be avoided by regular cleaning and sterilization of the sample chamber as described in the manual. We recommend this procedure be repeated every month when measuring glutamine in the 2900.

For further information, please contact:

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YSI Life Sciences develops and manufactures scientific instruments, sensors and systems that serve a variety of scientific and industrial markets worldwide. YSI has a long history in the life sciences and bioanalytical markets, most notably with our introduction of the world's first commercial whole blood glucose analyzer in 1975. Today there are over 10,000 YSI instruments installed around the world, trusted in critical situations to provide the most accurate data in the shortest time.

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