

## Simultaneous Measurement of Glutamine and Glutamate

### I. Introduction

Glutamine and Glutamate concentrations in complex matrices can be measured using the YSI 2700 SELECT 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 Dual-Channel 2700 SELECT 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,  $\alpha$ -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 to at both the glutamate and glutamine membranes, producing hydrogen peroxide,  $\alpha$ -ketoglutarate, and ammonia. The 2700 SELECT calculates the the glutamine concentration by subtracting the glutamate response from the glutamine response. In this way both glutamate and glutamine can be measured simultaneously.

### II. Material and Setup

- A. YSI 2700 SELECT 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 III Method C of this note.
- C. Connect the 2700 SELECT to a suitable power source.
- D. Perform the instrument and membrane check described in the Operations Manual (Section 3).
- E. Volumetric glassware (Class A recommended).
- F. The following instrument set up is recommended:

Sample size:	20 $\mu$ L***
Sample Station #	3, 4 or 5
CalMethod	Two Station

### Black Probe Parameters

Chemistry	Glutamate
Unit	mmol /L
Calibrator	5.00 mmol /L
End Point	30 Sec
CalStation#	1

### White Probe Parameters

Chemistry	Glutamine
Unit	mmol /L
Calibrator	5.00 mmol /L
End Point	30 Sec
CalStation#	2

### Autocal Parameters

Sample Error	ON
Temperature	1°C
Time	15 Min
Sample	1 Sam
Cal Shift	2%

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

### III. 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. Install the 5.00 mmol/L glutamate standard at station #1. Pour 5.00 mmol/L glutamine standard into a test tube and install at station #2. Calibrate the 2700 SELECT with 5.00 mmol/L glutamate and 5.00 mmol/L glutamine calibration standards.
- D. Check the linearity of the membrane at least once a day by injection of glutamate (10 mmol/L) and glutamine (8 mmol/L) linearity check solutions. Refer to the Operations Manual (Section 3) for specifications.
- E. Assay the sample by aspiration into the 2700 SELECT. The linear range of the system is 0 to 8.0 mmol/L glutamine, and 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 (Section 6).

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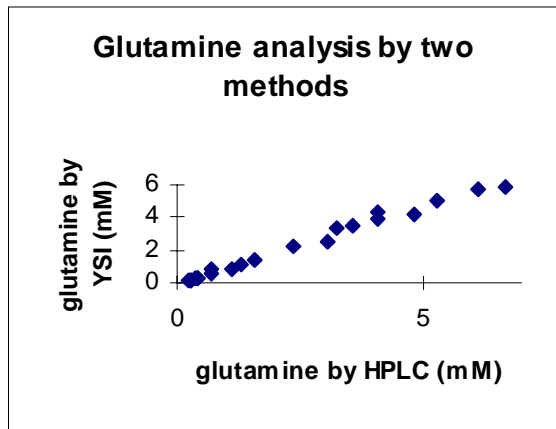
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#### IV. Results

The graph below is the result of testing the YSI 2700 SELECT vs. an HPLC for glutamine concentration, in cell culture media. (Data courtesy of a well established Biotechnology company.)



Slope 0.94,  $r^2 = 0.99$

#### V. Ordering Information

YSI No.

- 2700 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 2700 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 2700.

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