Diazyme Laboratories
12889 Gregg Court
Poway, CA 92064, USA
Tel: 858-455-4768 / Fax: 858-455-3701
Email **support@diazyme.com**
Website: www.diazyme.com

# Liquid Stable Lithium Enzymatic Assay

## Configuration

The Diazyme Liquid Stable Lithium Enzymatic Assay reagent is provided in bulk and in the following kit configurations:

| REF | Kit size |
|---|---|
| DZ116B-K | R1: 2 x 20 mL<br>R2: 2 x 10 mL<br>Cal: 3 x 3 mL |

## Indications for Use

Diazyme Lithium Enzymatic Assay kit is for quantitative *in vitro* determination of lithium in human serum. Measurements of Lithium are carried out essentially to ensure that proper drug dosage is administered in the treatment of patient suffering from bipolar disorder and to avoid toxicity.

## Clinical Significance [2,3]

For decades, lithium carbonate has remained one of the most effective agents for treatment of patients suffering from bipolar disorder (manic depressive psychosis). Lithium acts by altering intraneuronal metabolism of catecholamines, inhibition of noradrenaline sensitive adenylate cyclase, and reduction in synaptic transmission and increase in neuronal excitability with modification of central nervous system (CNS) amine levels. Recently, studies have also shown that lithium holds promise against Alzheimer's disease. However, lithium has many side effects. Over dosage of lithium can cause acute $Li^+$ intoxication, which occurs quite often due to its narrow therapeutic index. For example, serum $Li^+$ levels over 1.5mM (12 hours after a dose) usually indicate a significant risk of intoxication. Therefore, the timely and accurate monitoring of serum levels of lithium after a therapeutic dosage is critical.

## Assay Principle

Lithium is determined spectrophotometrically through a kinetic coupled enzyme assay system involving Diazyme's proprietary phosphatase[1] whose activity is sensitive to lithium (IC50=0.1mM). Through enzymatic coupling, the phosphatase substrate is converted to hypoxanthine by a series of enzymatic reactions to generate uric acid and hydrogen peroxide ($H_2O_2$). $H_2O_2$ generated reacts with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to form a quinone dye which has maximal absorbance at 556 nm. The rate of the quinine dye formation is inversely proportional to the concentration of lithium in serum samples. The coupled enzyme assay reaction scheme is as follows:

$$\text{Substrate} + H_2O \xrightarrow{\text{Phosphatase}} \text{AMP} + \text{Pi}$$

$$\text{AMP} + 2H_2O + \text{Pi} + 2O_2 \xrightarrow{\text{5'-NT/ADA/PNP/XOD}} \text{Pi} + NH_3 + \text{R-1-P} + \text{Uric Acid} + 2H_2O_2$$

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{Peroxidase}} 4H_2O + \text{Quinone dye}\ (\lambda\ \text{max}\ 556\ \text{nm})$$

*5'-NT: 5'-Nucleotidase*
*ADA: Adenosine Deaminase*
*PNP: Purine Nucleoside Phosphorylase*
*XOD: Xanthine Oxidase*
*AMP: Adenosine-5'-phosphate*
*R-1-P: Ribose-1-phosphate*

## Reagents – "Working Solutions"

REAGENT 1: Enzymes/substrates liquid containing Good's buffer, phosphatase substrate, 4-AA, enzymes and stabilizers

REAGENT 2: Enzymes/substrate liquid containing Good's buffer, enzymes, EHSPT, $MgCl_2$, and stabilizers

## Precautions

**DO NOT INGEST**. Avoid contact with skin and eyes. CONTROLS are human serum based. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

## Reagent Handling

The Diazyme Liquid Lithium Assay REAGENTS are ready-to-use. The CONTROLS are provided in lyophilized form. Reconstitute CONTROLS carefully according to instructions on accompanying lot-specific information literature. Refer to the CONTROLS package insert for preparation.

## Reagent Stability and Storage

The REAGENTS, CALIBRATORS, and CONTROLS are stable when stored as instructed until the expiration date on the label when stored at 2-8°C. Validation studies using the Hitachi 717 chemistry analyzer have shown the reagents to be stable for at least 12 months from the date of manufacture. REAGENTS stability on-board the Hitachi 717 instrument was determined to be at least 1 month.

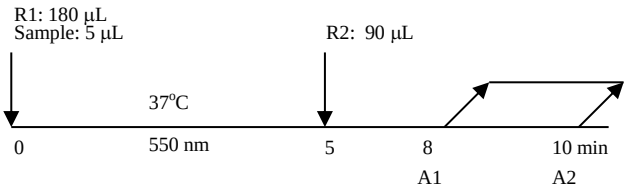
## Specimen Collection and Handling [2,3]

The assay is formulated for use with non-hemolyzed serum. No special handling or pretreatment is needed. It is recommended that a standardized 12-hour post dose serum lithium concentration be used to assess adequate therapy. Serum samples should be collected such that testing may be performed as soon as possible after the specimen collection.

## Materials Provided
See "Reagents – 'Working Solutions'" Section.

## Materials Required But Not Provided
An analyzer capable of dispensing 2 REAGENTS and of measuring absorbance at 550 nm with temperature control (37°C). Good laboratory practice recommends the use of control materials.

## Automated Chemistry Analyzer Assay Scheme




Application sheets (parameters) for use of Diazyme Liquid Stable Lithium Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

## Calibration
This assay should be calibrated every two weeks using low, medium and high lithium CALIBRATORS on the Hitachi 917 chemistry analyzer.

## Quality Control
Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guideline concerning the running of external quality control. Diazyme Lithium Normal and Abnormal CONTROLS (REF DZ116B-CON) are available separately.

To ensure adequate quality control, normal and abnormal CONTROL with known values should be run as unknown samples.

## Results
Results are printed out in mmol/L. Note: Samples with values greater than 3.0 mmol/L should be diluted with an equal part of 0.9% saline (1:1) and rerun. Multiply results by 2.

## Reference Range [2, 3, 4]
A trough concentration for 12 hour post dose is expected to be 1.0-1.2 mM. Levels higher than 1.5 mM, 12 hours after a dose, indicate a significant risk of intoxication. Values indicated should be used only as a guide. It is recommended that each laboratory establishes or derives a reference interval for the population it serves.

## Limitations
1. The assay is designed for use with human serum samples only.
2. There is a possibility that technical or procedural errors as well as other substances or factors not listed may interfere with the test and cause false results.

## Performance Characteristics
All performance studies were conducted using the Hitachi 717 automated chemistry analyzer.

### Accuracy

| | Diazyme Liquid Stable *vs* Diazyme Lyophilized (Hitachi 717 analyzer) | Diazyme Liquid Stable *vs* ISE |
|---|---|---|
| N | 62 | 62 |
| $r^2$ | 0.987 | 0.99 |
| Slope | 0.932 | 1.03 |
| y intercept | 0.088 | -0.04 |

### Precision
Within run

| | 1.0 mM Li+ (10 days, n=4) | 2.5 mM Li+ (10 days, n=4) |
|---|---|---|
| Mean | 0.97 mM | 2.50 mM |
| CV% | 4.3% | 1.2% |

Total

| | 1.0 mM Li+ (10 days, n=4) | 2.5 mM Li+ (10 days, n=4) |
|---|---|---|
| Mean | 0.97 mM | 2.50 mM |
| CV% | 4.8% | 1.3% |

### Linearity
The assay has a measuring range from 0.19 mmol/L-3.0 mmol/L

### Detection Limits
Testing per EP 17-A guidelines indicates Limit of Blank (LOB) value of 0.02 mM; Limit of Detection (LOD) value of 0.051 mM and Limit of Quantitation (LOQ) value of 0.19 mM lithium.

### Interference
The assay is not interfered by the following substances at indicated concentrations: $Na^+$ 200 mM, $NH_4^+$ 0.5 mM, $Ca^{2+}$ 4.0 mM, $Mg^{2+}$ 2.0 mM, ascorbic acid 5.0 mM, 0.25 mM $Zn^{2+}$, 0.25 mM $Fe^{3+}$, 0.25 mM $Cu^{2+}$, 10 mM $K^+$, Triglycerides 1000 mg/dL, conjugated bilirubin 20 mg/dL and unconjugated bilirubin 45 mg/dL, Ascorbic Acid 5 mM and hemoglobin 500 mg/dL.

### References

1. J.R. Murguia, J.M. Belles and R. Serrano A salt-sensitive 3'(2'),5'-bisphosphate nucleotidase involved in sulfate activation (1995), Science 267: 232-234
2. T.P. Moyer and C.E. Pippenger, Therapeutic Drug Monitoring, Tietz Textbook of Clinical Chemistry. CA C.A. Burtis and E.R. Ashwood eds, (Second ed.) W.B. Saunders Company.
3. A. Amdisen, Serum Lithium Determinations for Clinical Use (1967), Scand. J. Clin. Lab Invest. 20:104-108.
4. M.S. Wachtel *et al.*., Creation and Verification of Reference Intervals (1995), Laboratory Medicine 26: 593-597.



CE EC REP **MDSS**
**Schiffgraben 41**
**30175 Hannover,**
**Germany**

IVD
**12889 Gregg Court**
**Poway, CA 92064, USA**
**Tel: (858) 455-4754**
**Fax: (858) 455-4750**