

## Instruction manual

- \* FOR RESEARCH USE ONLY
- \* STORE AT 4°C UPON ARRIVAL

### Lithium (Li) Assay Kit LS (Polyfluoroporphyrin Chromogenic method)

#### Description

Lithium carbonate used in the treatment of bipolar disorder. Lithium carbonate is absorbed by a gastrointestinal. The overdose of a lithium is occur nephropathy. Therefore and this medication manage are required. Serum lithium levels higher than 1.5 mM indicate a significant risk of intoxication.

This Lithium reagent kit is a chromogenic method.

Lithium present in the serum sample give a magenta colored complex with polyfluoroporphyrin (as chromogen). The color intensity is proportional to the amount of lithium present in the sample. This product can perform stable assay under ordinary temperature and atmospheric pressure. No need gas barrier function enable us to use all type of measuring equipment including existing autoanalyzer. Excellent storage stability (even after opening sealed package) gives extended validated date.

\*Patent number. JP 5100903, JP 5222432

#### Operation

##### 1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation.

##### 2. Assay preparation

Bring all reagents to room temperature before use.

#### Kit contents

100 tests (Catalog # : LI01ME)

R-R Chelate color	●	24 mL×1
( Polyfluoroporphyrin )		
STD Lithium Standard 2.0 mM	●	0.4 mL×1

#### Note

- A) Unstableness of incubation temperature may result in unstable results.
- B) Use disposable test tube and glassware washed with 1M HNO<sub>3</sub> or 1M HCl solution and distilled water.
- C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and reagents must be poured accurately μL level.
- D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.
- E) Heparin lithium anticoagulant affects to chromogenic system. The best samples are EDTA plasma or citric acid plasma, and non-hemolyzed serum.

### 3. Assay procedure.

#### Procedure using microplate reader.

(1 assay sample 244 µL)

#### ○Assay

- Add 4 µL of Distilled water (Blank) / STD (Standard)/ sample into each well.
- Add 240 µL of R-R to each well and incubate at room temperature for 10 min.
- Read the absorbance at 550 nm (main) and 600 nm (sub).  
--> OD

\*Select the filter: 540-560 nm at 550 nm, 600-610 nm at 600 nm.

		Assay Sample		
Add	(µL)	Blank OD <sub>BI</sub>	Standard OD <sub>Std</sub>	Sample OD <sub>S</sub>
1	Distilled water	4	-	-
	STD	-	4	-
	Assay sample	-	-	4
2	R-R	240	240	240

↓

Mix and incubate for 10 minutes at room temperature.  
Read the absorbance at 550 nm (main) and 600 nm (sub).  
(Possible ranges of wavelength for selects the filter: 540-560 nm at 550 nm, 600-610 nm at 600 nm. )

#### ○Calculations

$$\Delta OD_{Std} = OD_{Std} - OD_{BI}$$

$$\Delta OD_S = OD_S - OD_{BI}$$

$$\text{Lithium (mg/dL)} = \Delta OD_S / \Delta OD_{Std} \times 1.39$$

$$\text{Lithium (mM)} = \Delta OD_S / \Delta OD_{Std} \times 2.0$$

(Assay example)

	OD (550 nm)	OD (600 nm)	OD	ΔOD	Lithium (mmol/L)
Blank	0.219	0.207	0.012	-	-
Standard	0.573	0.192	0.381	0.369	-
Sample	0.378	0.204	0.174	0.162	0.88

#### \*Observed 550 nm with 600 nm

$$[OD = OD(550 \text{ nm}) - OD(600 \text{ nm})]$$

$$\Delta OD_{Std} = (0.573 - 0.192) - (0.219 - 0.207) = 0.369$$

$$\Delta OD_S = (0.378 - 0.204) - (0.219 - 0.207) = 0.162$$

$$\begin{aligned} \text{Lithium}_{\text{Sample}} (\text{mg/dL}) &= \Delta OD_S / \Delta OD_{Std} \times 1.39 \\ &= 0.162 / 0.369 \times 1.39 = 0.609 (\text{mg/dL}) \end{aligned}$$

$$\begin{aligned} \text{Lithium}_{\text{Sample}} (\text{mM}) &= \Delta OD_S / \Delta OD_{Std} \times 2.0 \\ &= 0.162 / 0.369 \times 2.0 = 0.88 (\text{mM}) \end{aligned}$$

#### \*Observed 550 nm only

$$[OD = OD(550 \text{ nm})]$$

$$\Delta OD_{Std} = 0.573 - 0.219 = 0.354$$

$$\Delta OD_S = 0.378 - 0.219 = 0.159$$

$$\begin{aligned} \text{Lithium}_{\text{Sample}} (\text{mg/dL}) &= \Delta OD_S / \Delta OD_{Std} \times 1.39 \\ &= 0.159 / 0.354 \times 1.39 = 0.623 (\text{mg/dL}) \end{aligned}$$

$$\begin{aligned} \text{Lithium}_{\text{Sample}} (\text{mM}) &= \Delta OD_S / \Delta OD_{Std} \times 2.0 \\ &= 0.159 / 0.354 \times 2.0 = 0.90 (\text{mM}) \end{aligned}$$

\*In diluted sample of seminal fluid, multiply the result by dilution-factor.

#### Performance

Measuring range 0.03 – 3.0 mM  
Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run			
	Mean mM	S.D	C.V %
Level 1	0.72	0.03	3.26
Level 2	1.47	0.04	2.68

Interferences No interference by the note of substances were observed.  
[Observed 500nm only]  
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL  
Hemoglobin 300 mg/dL Chyle 300FTU  
[Observed 500nm with 600nm]  
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL  
Hemoglobin 1 g/dL Chyle 3,000 FTU

#### Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.  
Expiration: 1 year from the date of manufacture.  
After the bottles are opened,  
the kit should be used in 1 month.

#### Reference

- Synthesis of F28 tetraphenylporphyrin and its application to the separation and detection of lithium( I ).  
Kenji KOYANAGI, Masaaki TABATA, BUNSEKI KAGAKU;  
ISSN:0525-1931;vol.51, No.9, pp803-807(2002)
- Patent number. JP 5100903, JP 5222432

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\*Metallogenics™ is the name of reagent kit from Cellspect Co., Ltd.