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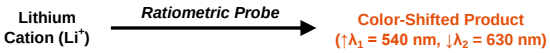
Lithium Assay Kit (Colorimetric)

9/17

(Catalog # K545-100; 100 Reactions; Store at -20°C)

I. Introduction:

Lithium, the lightest alkali metal element, is a ductile, reactive metal that occurs in nature as the Li^+ cation and is found in various mineral compounds. Physiologically, lithium is present only in trace levels and is not considered to be an essential dietary nutrient. However, lithium is routinely used in medicine as a psychoactive drug. Lithium acts as a mood stabilizer and is considered to be the gold standard first-line treatment for bipolar depression and acute mania. Patients treated with lithium are less likely to require hospitalization or complete suicide than patients treated with other mood stabilizers or antipsychotics. Chemically, lithium is the simplest possible drug—as an element, it is not metabolized by the body and has zero-order renal elimination kinetics. Lithium has a narrow therapeutic/toxic ratio and requires blood level monitoring, both to ensure efficacy and decrease the risk of toxic side-effects. The target serum concentration for lithium maintenance therapy ranges from 0.6-1.2 mM. At serum levels ≥1.5 mM there is a sharp increase in severe adverse effects. Overt, potentially fatal side effects (such as seizures, ataxia and loss of consciousness) are observed at serum levels >2.5 mM. BioVision's Lithium Assay Kit allows for quantification of lithium levels in biological fluids such as serum and plasma. The assay uses a lithium-selective bichromatic probe that undergoes an absorbance change at two distinct wavelengths upon binding to Li^+ (λ_1 = 540 nm, λ_2 = 630 nm). The ratio of the two optical measurements is used to accurately calculate sample Li^+ concentration. The kit also includes a sodium masking reagent to prevent the possibility of interference by supra-physiological levels of serum Na^+ (hypernatremia). The assay is quick, is high-throughput adaptable and has a linear range from 0.5 – 10 nmole lithium per well (corresponding to 0.1 – 2 mM serum lithium).



II. Applications:

- Estimation of lithium concentration in various biological fluids

III. Sample Type:

- Human biological fluids (serum/plasma, *etc*.)

IV. Kit Contents:

Components	K545-100	Cap Code	Part Number
Lithium Assay Buffer	25 ml	WM	K545-100-1
Probe Solution	10 ml	NM	K545-100-2
Sodium-Masking Solution	1.5 ml	White	K545-100-3
Lithium Standard (10 mM)	500 µl	Yellow	K545-100-4

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 540 nm and 630 nm)
- Clear 96-well plates with flat bottom

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Lithium Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

- **Probe Solution:** Provided as a ready-to-use solution. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature and vortex thoroughly.
- **Sodium-Masking Solution:** Provided as a ready-to-use solution. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature and vortex thoroughly.
- **Lithium Standard (10 mM):** Provided as a 10 mM stock solution of LiCl in ddH_2O. Store at -20°C. Prior to use, warm solution to room temperature and vortex thoroughly.

VII. Lithium Assay Protocol:

1. Sample Preparation:

a. Collect serum or plasma samples by standard methods (see note regarding compatible blood collection tubes below). Samples exhibiting lipemia or excessive turbidity should be clarified by centrifugation at 10,000 x *g* for 5 min in order to separate lipid globules.

b. Add 5 µl of undiluted serum/plasma sample to desired well(s) in a clear, flat bottom 96-well plate.

c. To each sample well, add 15 µl of the Sodium-Masking Solution, bringing the volume up to 20 µl per well.

Notes:

- For blood lithium level determination, we recommend using serum collected in tubes that are free of additives or preservatives ("off-the-clot" serum). If plasma is used, it should be collected in K_2EDTA-coated tubes that are free of lithium- or sodium-based anticoagulants or preservatives (*e.g.* lithium/sodium heparin, sodium citrate), as these additives will interfere with the assay.
- For unknown samples, we recommend performing a pilot experiment to ensure readings are within the standard curve range. Samples that are outside of the standard curve range may be diluted with ddH_2O and retested (in this case, use 5 µl of the pre-diluted sample and add 15 µl Sodium-Masking Solution to each well).

2. Standard Curve Preparation: Prepare a 500 µM solution of lithium by adding 50 µl of the 10 mM Lithium Standard to 950 µl of dH_2O. Add 0, 4, 8, 12, 16, and 20 µl of the 500 µM working solution into a series of wells, generating 0, 2, 4, 6, 8 and 10 nmol of lithium/well. Adjust the volume of all of the standard curve wells (including the 0 nmol/well reagent blank) to 20 µl/well with ddH_2O.

Note: To ensure accurate quantification of lithium in samples, a standard curve should be prepared each time the assay is performed.

3. **Reaction Preparation:**
   - **a.** Add 130 µl of Lithium Assay Buffer to all sample and standard curve wells.
   - **b.** Add 100 µl of Probe Solution to all sample and standard curve wells (bringing the final volume to 250 µl per well).
   - **c.** Incubate the plate at room temperature for 5 min with gentle orbital shaking to ensure well contents are effectively mixed.
4. **Measurement:** Measure the absorbance of all sample and standard curve wells at both 540 nm and 630 nm in endpoint mode.
5. **Calculations:** For all standard curve and test sample wells, calculate the absorbance ratio (A_{ratio}) by dividing the well OD_{540} value by the OD_{630} value ($A_{ratio} = OD_{540}/OD_{630}$). For the Lithium Standard curve, subtract the absorbance ratio obtained for the reagent blank (0 nmol/well standard) from all of the standard absorbance ratios, plot the background-subtracted A_{ratio} values and calculate the slope of the standard curve. For test samples, calculate the corrected sample absorbance ratio (A_C) by subtracting the reagent blank (0 nmol/well standard) from the calculated sample ratio: $A_C = (OD_{540}/OD_{630})_{sample} - (OD_{540}/OD_{630})_{blank}$. Apply the A_C values to the standard curve to get *B* nmol of lithium in the well.

$$\textbf{Sample Lithium Concentration} = \frac{B}{V} \times D = \textbf{nmol/µl} \equiv \textbf{mM}$$

Where: ***B*** is the amount of lithium, calculated from the standard curve (in nmol)
V is the volume of sample added to the well (5 µl)
D is the sample dilution factor (if applicable, *D*=1 for undiluted samples)

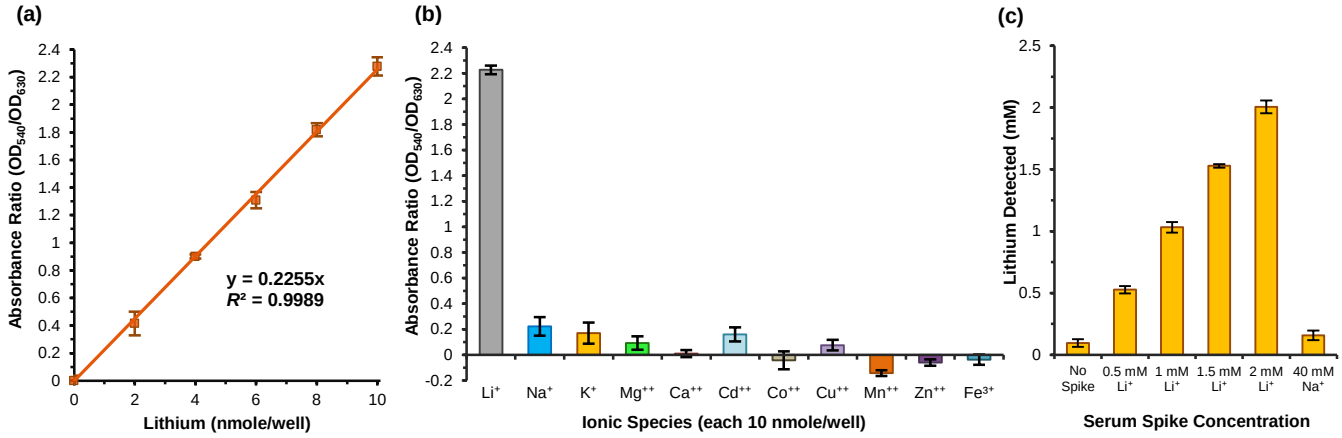


Figure: (a) Lithium Standard curve. Lithium concentration is directly proportional to the ratio of absorbance measured at 540 nm and 630 nm. **(b)** Specificity for detection of lithium (Li^+) over other common monovalent, divalent and trivalent ions (each 10 nmole/well). All other cations tested contribute ≤10% interference when normalized to the signal generated by 10 nmole lithium. **(c)** Estimation of lithium in human serum. Normal "off-the-clot" pooled serum (5 µl, undiluted) was spiked with 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM lithium standard. Mean lithium concentrations detected in the spiked samples were 0.52 mM, 1.03 mM, 1.53 mM and 2.01 mM, respectively (mean spike recovery rates across all spiked concentrations ranged from 100.3 – 105.5%). Potential interference by excessive serum sodium (hypernatremia) was also tested. Normal human serum (5 µl, undiluted) was spiked with an additional 40 mM NaCl to simulate hypernatremic conditions (serum Na^+ ≥175 mM). The signal imparted by the additional 40 mM Na^+ was equivalent to that of 0.062 mM lithium (≤10% interference for the typical therapeutic range). Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.

VIII. RELATED PRODUCTS:

Sodium Assay Kit (K391)	Zinc Fluorometric Assay Kit (K428)	EZDetect™ Lithium Probe (B1548)
Calcium Assay Kit (K380)	Chloride Assay Kit (K530)	Cobalt Assay Kit (K505)
Iron Assay Kit (K390)	Magnesium Assay Kit (K385)	Nickel Assay Kit (K510)

FOR RESEARCH USE ONLY! Not to be used on humans.