3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Lithium Assay Kit

Catalog Number **MAK358**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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.

The 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).

The kit is suitable for the determination of lithium concentration in biological samples such as serum and plasma.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Lithium Assay Buffer Catalog Number MAK358A	25 mL
Probe Solution Catalog Number MAK358B	10 mL
Sodium-Masking Solution Catalog Number MAK358C	1.5 mL
Lithium Standard (10 mM) Catalog Number MAK358D	500 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well flat-bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader
- Orbital shaker

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

Lithium Assay Buffer – Warm to room temperature prior to use.

Probe Solution and Sodium-Masking Solution – Provided as ready-to-use solutions. Divide into aliquots and store at –20 °C, protected from light. Prior to use, warm solutions to room temperature and vortex thoroughly.

Lithium Standard (10 mM) – Provided as a 10 mM stock solution of LiCl in ultrapure water. Store at –20 °C. Prior to use, warm solution to room temperature and vortex thoroughly.

Procedure

Sample Collection

For blood lithium level determination, use 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.

Sample Preparation

1. Collect serum or plasma samples by standard methods (as described in Sample Collection section).
2. Samples exhibiting lipemia or excessive turbidity should be clarified by centrifugation at 10,000 × *g* for 5 minutes to separate lipid globules.
3. Add 5 µL of undiluted serum or plasma sample to desired well(s) in a clear, flat bottom 96 well plate.
4. To each sample well, add 15 µL of the Sodium-Masking Solution, bringing the volume up to 20 µL per well.
5. For unknown samples, it is recommended to perform 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 ultrapure water and retested (in this case, use 5 µL of the prediluted sample and add 15 µL of Sodium-Masking Solution to each well).

Standard Curve Preparation

To ensure accurate quantification of lithium in samples, a standard curve should be prepared each time the assay is performed. Prepare a 500 µM solution of lithium by adding 50 µL of the 10 mM Lithium Standard to 950 µL of ultrapure water. Prepare Lithium Standards in desired wells of a clear 96 well plate according to Table 1. Mix well.

Table 1.
Preparation of Lithium Standards

Well	500 µM Premix	Ultrapure Water	Lithium (nmol/well)
1	0 µL	20 µL	0
2	4 µL	16 µL	2
3	8 µL	12 µL	4
4	12 µL	8 µL	6
5	16 µL	4 µL	8
6	20 µL	–	10

Assay Reaction

1. Add 130 µL of Lithium Assay Buffer to all sample and standard curve wells.
2. Add 100 µL of Probe Solution to all sample and standard curve wells (bringing the final volume to 250 µL per well).
3. Incubate the plate at room temperature for 5 minutes with gentle orbital shaking to ensure well contents are effectively mixed.

Measurement

Measure the absorbance of all sample and standard curve wells at both 540 nm (A_{540}) and 630 nm (A_{630}) in endpoint mode.

Results

1. For all standard curve and test sample wells, calculate the absorbance ratio (A_{ratio}) by dividing the well A_{540} value by the A_{630} value ($A_{ratio} = A_{540}/A_{630}$).
2. 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.
3. 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:

$$A_C = [(A_{540}/A_{630})_{sample}] - [(A_{540}/A_{630})_{blank}]$$

Apply the A_C values to the standard curve to get B nmol of lithium in the well.

$$\text{Lithium concentration (nmol/µL or mM)} = (B/V) \times D$$

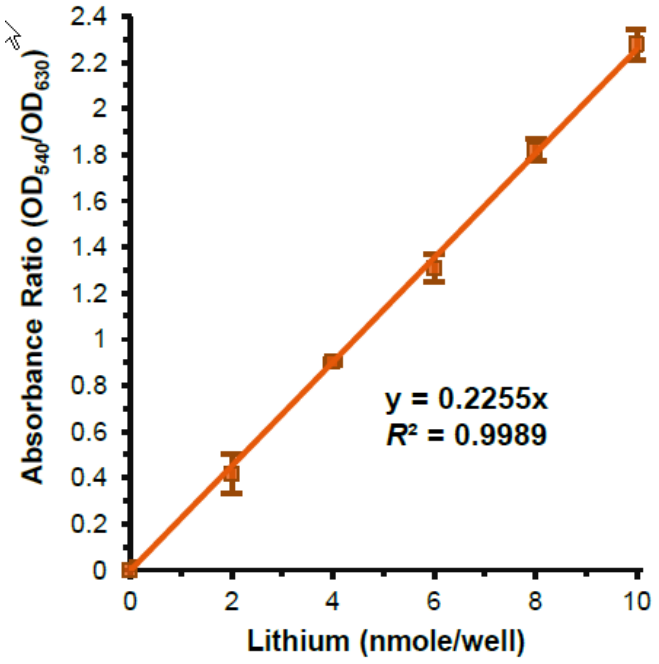
where:

B = the amount of lithium, calculated from the standard curve (in nmol)

V = the volume of sample added to the well (5 µL)

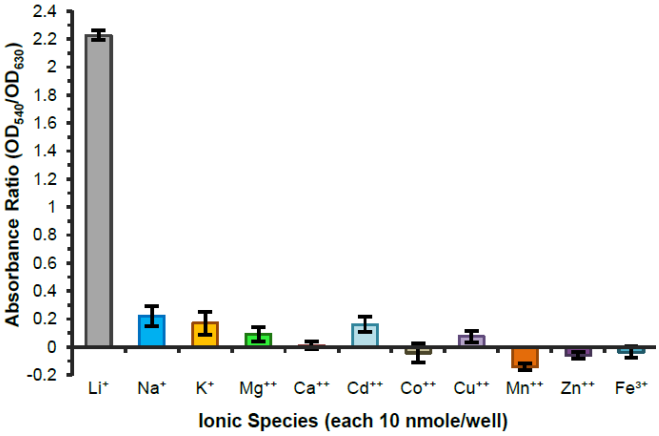
D = the sample dilution factor (D = 1 for undiluted samples)

Figure 1.
Typical Lithium Standard Curve



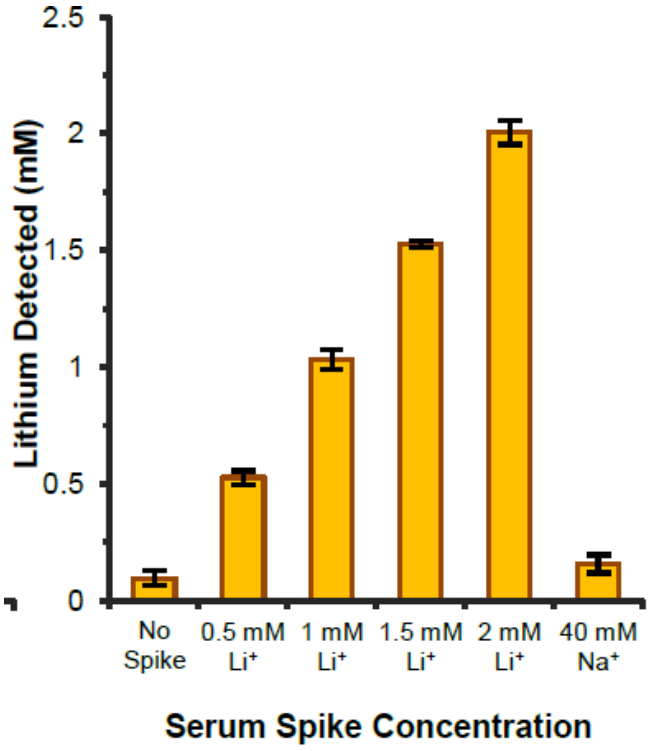
Lithium concentration is directly proportional to the ratio of absorbance measured at 540 nm (A_{540}) and 630 nm (A_{630}).

Figure 2.
Assay Specificity



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.

Figure 3.
Estimation of Lithium in Human Serum



Normal "off-the-clot" pooled serum (5 µL and 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 and 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 procedure.

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