Measure-iT™ Lead and Cadmium Assay Kit (M36353)

Quick Facts

Storage upon receipt:

- Store at ≤–20ºC
- Protect from light
- Desiccate
- Avoid freeze/thaw cycles

Ex/Em: 495/516 nm

Number of assays: 1000, when using a 200 µL assay volume

Introduction

The Measure-iT™ Lead and Cadmium Assay Kit provides easy and accurate quantitation of lead or cadmium. The kit supplies concentrated assay reagent, dilution buffer, and concentrated lead and cadmium standards. Simply dilute the reagent 1:200, load 200 µL into the wells of a microplate, add 1–20 µL sample volumes, mix, then read the fluorescence (Figure 1). The assay has a linear range of 5–200 nM of either lead or cadmium. The assay is performed at room temperature, and the signal is stable for at least 30 minutes. Cross-contamination by many metals is well tolerated.

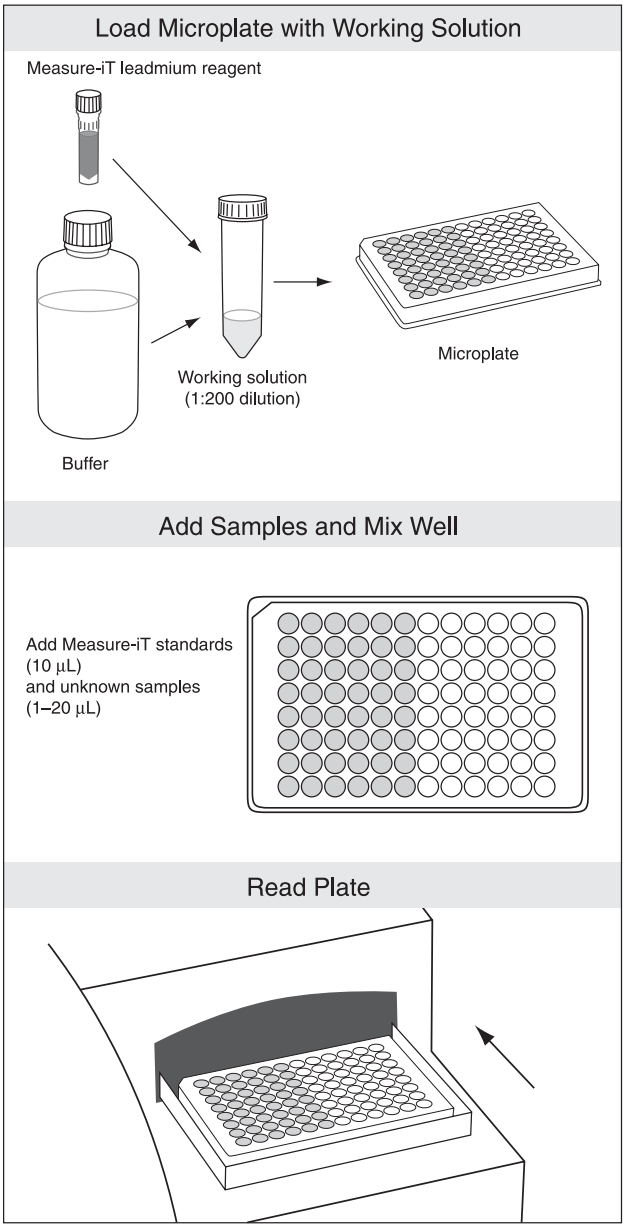

Figure 1. The Measure-iT™ Lead and Cadmium Assay.

Materials

Kit Contents

- **Component A:** Measure-iT™ Leadmium reagent, 1.0 mL of a 200X concentrate in DMSO
- **Component B:** Measure-iT™ Leadmium buffer, 20 mL of a 10X concentrate
- **Component C:** Lead standard, 5.0 mL of 8.4 µM in deionized H_2O
- **Component D:** Cadmium standard, 5.0 mL of 8.4 µM in deionized H_2O

The kit provides sufficient material for 1000 assays.

Storage

Upon receipt, store the kits at ≤–20ºC, desiccated and protected from light. Under these conditions the components should be stable for at least 6 months. For convenience, the Measure-iT™ Leadmium reagent (Component A) may be stored short term (days) at room temperature, protected from light. The Measure-iT™ Leadmium buffer (Component B) may also be stored short term (days) at room temperature; however, for longer periods, we recommend storage at ≤–20°C to prevent microbial contamination.

Table 1. Preparation of Measure-iT™ Lead or Cadmium Standards.

Concentration of Lead or Cadmium Standard (nM)	Volume of Component C or D (µL)	Volume of Deionized H_2O (µL)
0	0	1000
210	25	975
420	50	950
840	100	900
1680	200	800
2520	300	700
3360	400	600
4200	500	500

Handling and Disposal

We must caution that no data are available addressing the toxicity of the Measure-iT™ Leadmium reagent. This reagent is known to bind metal ions and is provided as a solution in DMSO; treat the reagent with the same safety precautions as all other chemicals with unknown toxicity, and dispose in accordance with local regulations.

Experimental Protocol

General Considerations

During all steps, protect the Measure-iT™ Leadmium reagent concentrate and the working solution from light as much as possible. Allow the kit components to equilibrate to room temperature before use. The assay temperature is "room temperature," defined here as 20–25°C. Assay temperatures outside this range have not been tested but may be acceptable.

Lead or Cadmium Assay Procedure

1. Prepare Measure-iT™ lead or cadmium standard dilutions from the provided concentrate. Dilute the provided lead (Component C) or cadmium (Component D) standard with deionized H_2O according to Table 1. The standards are stable for at least 2 years when stored at ≤–20ºC.

2. Dilute the Measure-iT™ Leadmium buffer concentrate (Component B) 10-fold in deionized H_2O. For example, to prepare enough 1X buffer for ~100 assays, dilute 2 mL Component B into 18 mL deionized H_2O. Buffer concentrate may also be diluted directly along with the reagent when making the 1X working solution (Step 3).

3. Make a working solution by diluting Measure-iT™ Leadmium reagent 1:200 in diluted 1X Measure-iT™ Leadmium buffer. For example, for ~100 assays put 100 µL of Measure-iT™ Leadmium reagent (Component A) and 20 mL of 1X buffer (from Step 2) in a disposable plastic container and mix well. Do not use glass containers.

4. Load 200 µL of the working solution into each microplate well. Diluted Measure-iT™ Leadmium reagent is stable (±10%) for at least 4 days at room temperature, protected from light.

5. Add 10 µL of each lead or cadmium standard prepared in Step 1 to separate wells and mix well. Duplicates or triplicates of the standards are recommended.

6. Add 1–20 µL of each unknown lead or cadmium sample to separate wells and mix well. Duplicates or triplicates of the unknown samples are recommended. Some contaminating metal ions may interfere with the assay (Table 2). For highest precision, the volumes of all reactions can be equalized by adding a small volume of the dilution buffer. Equalizing the volumes is important in cases where contaminating substances may be present.

7. Measure the fluorescence using a microplate reader (excitation/emission maxima are 490/520 nm). The maximum fluorescence signal is attained within one minute and is stable (±10%) for at least 30 minutes.

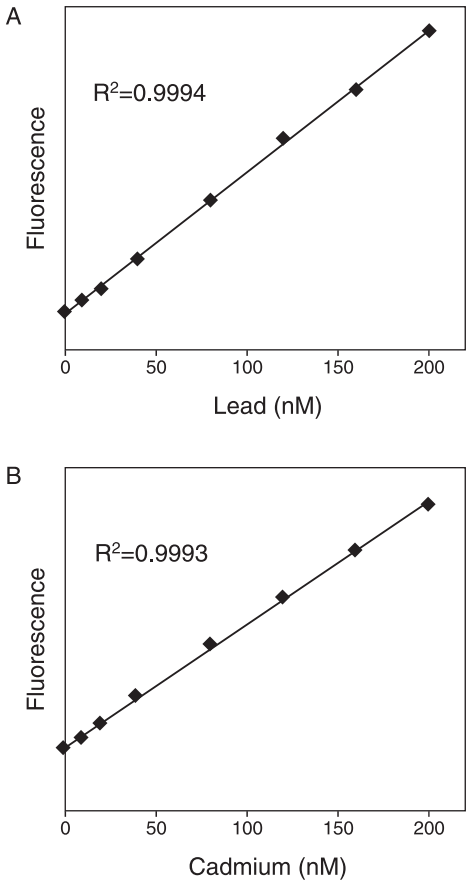

Figure 2. Linearity and sensitivity of the Measure-iT™ Lead and Cadmium Assay for lead (Panel A) and cadmium (Panel B). Triplicate 10 µL samples of lead and cadmium were assayed; fluorescence was measured at 490/520 nm and plotted versus lead or cadmium concentration. The variation (CV) of replicate samples was <2%.

Table 2. Effect of Contaminants in the Measure-iT™ Lead and Cadmium Assay. *

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Calcium	1 µM 5 µM	10 µM 50 µM	20 µM 100 µM	OK NR
Magnesium	500 µM	5 mM	10 mM	OK
Silver	100 µM	1 mM	2 mM	OK
Aluminum	10 µM	100 µM	200 µM	OK
Manganese	10 µM	100 µM	200 µM	OK †
Zinc	100 nM 500 nM	1 µM 5 µM	2 µM 10 µM	OK NR
Nickel	500 nM 1 µM	5 µM 10 µM	10 µM 20 µM	OK NR
Chromium (III)	500 nM 1 µM	5 µM 10 µM	10 µM 20 µM	OK NR
Copper	500 nM 1 µM	5 µM 10 µM	10 µM 20 µM	OK NR
Iron (III)	1 µM	10 µM	20 µM	OK
Cadmium	10 nM 100 nM	100 nM 1 µM	200 nM 2 µM	OK NR
Mercury	5 µM 10 µM	50 µM 100 µM	100 µM 200 µM	OK NR
Barium	10 µM 20 µM	100 µM 200 µM	200 µM 400 µM	OK NR
Arsenic	500 µM	5 mM	10 mM	OK
Potassium chloride	10 mM	100 mM	200 mM	OK
Sodium chloride	50 mM	500 mM	1 M	OK †
Lithium chloride	50 mM	500 mM	1 M	OK †
Glutathione	1µM	10 µM	20 µM	OK
Nitrate	200 µM	2 mM	4 mM	OK
Nitrite	500 µM	5 mM	10 mM	OK
Ethanol	0.1%	1%	2%	OK
Phenol	0.01%	0.1%	0.2%	OK
Toluene	0.1%	1%	2%	OK
Dichloromethane	0.1%	1%	2%	OK

***** Lead standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. Results are given either as OK = usually less than 10% perturbation, or as NR = not recommended. **†** An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples.

8. Use a standard curve to determine the unknown lead or cadmium concentration. For the lead or cadmium standards, plot the metal ion concentration vs. fluorescence and fit a straight line to the data points.

Protocol Details

Generating Standard Curves

In this manual we have plotted standard curves as concentration (nM) of lead or cadmium vs. fluorescence. Alternatively, the x-axis can be expressed in ppb or as the concentration of the added sample. Table 3 is provided to facilitate these unit conversions.

The assay is linear from 5–200 nM (Figure 2). For best results at the low end of the standard curve, the line should be forced through the background point (or through zero, if background has been subtracted). When 10 µL volumes of the prepared standards are used, the lowest lead- or cadmium-containing standard represents 10 nM of lead or cadmium; however, highly accurate determinations of lead or cadmium down to 5 nM can be attained using the standard curve as described above.

Table 3. Concentration Conversion.*

Final Concentration (nM)	ppb Lead	ppb Cadmium	Concentration (µM) in Given Sample Volume		
			1 µL	10 µL	20 µL
5	1	0.6	1	0.1	0.05
100	20	12	20	2	1
200	40	24	40	4	2

* The Measure-iT™ Lead and Cadmium assay is designed to detect 5–200 nM of lead or cadmium in a 200 µL assay volume. Sample volumes may vary from 1–20 µL; therefore, sample concentration may vary from 0.05–40 µM.

Contaminating Substances

A number of common contaminants have been tested in the presence of lead in the Measure-iT™ Lead and Cadmium assay, and most are well tolerated (Table 2). Tolerances are similar when cadmium is assayed. For untested contaminating substances, and for highest accuracy, the standards should be assayed under the same conditions as the unknowns. For example, if the experimental samples are in a non-standard buffer and if 10 µL volumes of these samples are used, add 10 µL volumes of the non-standard buffer (lacking lead or cadmium) when assaying the standards.

Excitation and Emission Maxima

The excitation and emission maxima for the Measure-iT™ Leadmium reagent bound to lead or cadmium are 495 and 516 nm, respectively (Figure 3). Excitation/emission settings of 490/520 nm work well in the assay.

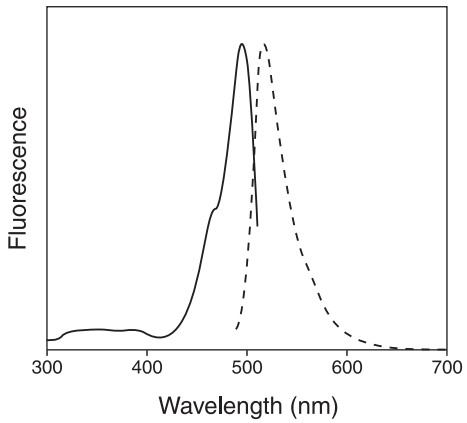

Figure 3. Normalized excitation and emission maxima for the Measure-iT™ Leadmium reagent bound to lead.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
M36353	Measure-iT™ Lead and Cadmium Assay Kit *1000 assays*	1 kit

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

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