Intracellular Lead and Cadmium Detection Kit for Flow Cytometry (I36352)

Quick Facts

Storage upon receipt:

- ≤–20°

Ex/Em:

- 490/520 nm for Leadmium™ Green dye
- 535/617 nm for propidium iodide bound to nucleic acids

Number of assays: 200 tests, based on a 1 mL assay volume

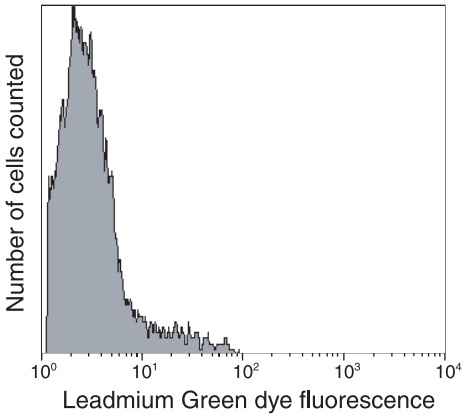

Figure 1. Background fluorescence of Leadmium™ Green dye in the Jurkat cell line. Cells in saline were loaded with Leadmium™ Green AM dye and washed. The single-color fluorescence was collected using 488 nm excitation and a 525/10 nm bandpass filter. Because no intracellular lead or cadmium was present in the cells, this histogram indicates the background fluorescence contributed by Leadmium™ Green dye.

Introduction

Heavy metals are ubiquitous environmental contaminants and exhibit widespread toxicity. Although standard assays exist for metal ions in solution, specific indicators to detect intracellular levels of lead or cadmium were not previously available. The Intracellular Lead and Cadmium Detection Kit employs the Leadmium™ Green AM dye as a specific indicator of lead or cadmium in cells. After the cells are loaded with dye, nonspecific esterases cleave the acetoxymethyl (AM) group, resulting in a charged form of the Leadmium™ Green dye that leaks out of cells far more slowly than the parent compound. The calcium-insensitive Leadmium™ Green dye becomes fluorescent in the presence of nanomolar levels of lead and micromolar levels of cadmium.

Product Information:

Components

- Leadmium™ Green AM probe (Component A), 50 µg in each of 5 vials
- $PbCl_2$ (Component B), 6 mL of a 10 µM solution in dH_20
- $CdCl_2$ (Component C), 6 mL of a 1 mM solution in dH_20
- Ionomycin (Component D), 500 µL of a 500 µM solution in DMSO
- TPEN (tetrakis-(2- pyridylmethyl) ethylenediamine) (Component E), 5 mg
- Propidium iodide (PI) (Component F), 1 mL of a 150 µM solution in dH_20
- Dimethylsulfoxide (DMSO) (Component G), 500 µL

Storage and Handling

Upon receipt, store kit at ≤–20°C. To minimize condensation, allow vials to warm to room temperature before opening. After Components B–D are thawed for the first time, it is permissible to store these tubes at 4°C; they should be stable for at least 6 months under these conditions.

Once prepared, solutions of the Leadmium™ Green AM dye in DMSO should be used within 1 day.

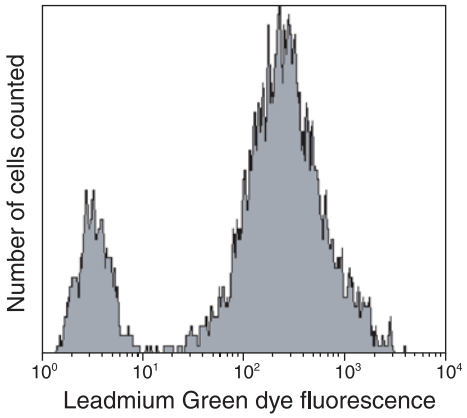

Figure 2. Fluorescence of Jurkat cells containing $PbCl_2$ in the Jurkat cell line. Cells in saline were loaded with Leadmium™ Green AM dye and washed and then incubated in the presence of 1 µM $PbCl_2$ in saline and 1 µM ionomycin. The single-color fluorescence was collected using 488 nm excitation and a 525/10 nm bandpass filter. The histogram shows cells positive for intracellular lead.

Spectral Characteristics

The approximate excitation and emission maxima of Leadmium™ Green dye are 490 nm and 520 nm, respectively. Cells labeled with the Leadmium™ Green dye can be analyzed by flow cytometry using 488 nm excitation and measuring fluorescence emission at 520 nm. Propidium iodide (PI) can be analyzed by flow cytometry using 488 nm excitation and fluorescence emission at 617 nm.

Before You Begin

Materials Required but Not Provided

- Saline (0.85% NaCl);
  Note: phosphate-buffered saline (PBS) should not be used
- Ethanol

Reagent Preparation

1.1 Remove one vial of Leadmium™ Green AM dye (Component A), and Components B, C, D, E, F, and G from freezer and let come to room temperature. Protect Component A from light.

1.2 Make the Leadmium™ Green AM stock solution by adding 50 µL DMSO (Component G) to one vial of dye (Component A, contains 50 µg). Mix well and protect from light.

1.3 Prepare a Leadmium™ Green AM dye working solution by diluting Leadmium™ Green AM stock solution (prepared in step 1.2) in saline 1:10 (i.e., adding 20 µL stock solution dye to 180 µL saline). Protect dye solutions from light at all times.

1.4 Warm saline to 37°C. This will be used for washing the samples between various steps.

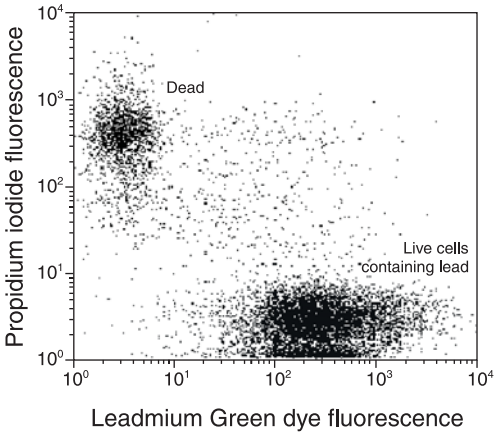

Figure 3. Dual-color scatter plot showing two populations. Jurkat cells were loaded with Leadmium™ Green AM dye and washed. The sample was then incubated in the presence of 1 µM $PbCl_2$ (in saline) and 1 µM ionomycin. After washing the sample, it was incubated in the presence of PI. Dual-color fluorescence was collected using 488 nm excitation and 525/10 nm and 610/10 nm bandpass filters. This results in visualization of two populations: dead cells (positive for PI) and live cells that contain lead (positive for Leadmium™ Green dye and negative for PI).

1.5 OPTIONAL: If the experiment will include comparative controls (treatment of cells with known concentrations of $PbCl_2$), a recommended starting concentration range for $PbCl_2$ is 625 nM down to 1 nM. Make 625 nM $PbCl_2$ by adding 250 µL of 10 µM $PbCl_2$ (Component B) to 3.75 mL saline. Make 9 further serial dilutions of this to arrive at 1 nM $PbCl_2$. Store these solutions at 37˚C until required. You will need 1 mL of each concentration for one treatment. Adjust the volumes transferred in the serial dilutions if replicates are desired.

1.6 OPTIONAL: If the experiment will include comparative controls (treatment of cells with known concentrations of $CdCl_2$), a recommended starting concentration range for $CdCl_2$ is 250 µM down to 1 µM. Make 250 µM $CdCl_2$ by adding 1 mL of 1 mM $CdCl_2$ (Component C) to 3 mL saline. Make 8 further serial dilutions of this to arrive at 1 µM $CdCl_2$. Store these solutions at 37˚C until required. You will need 1 mL of each concentration for one treatment. Adjust the volumes transferred in the serial dilutions if replicates are desired.

1.7 Make a 10 mM working solution of TPEN (Component E) solution by adding 1.2 mL of ethanol to the Component E vial. Mix well and store at room temperature.

Protocol

This protocol was optimized using Jurkat cells, a human T-cell line. To achieve optimal results, experimental parameters such as suspension buffers, media, and cell concentration should be adjusted depending on cell type and culture conditions used.

Note: Phosphate buffers should be avoided due to metal salt insolubilities.

Staining Cells

2.1 Harvest the cell sample(s) to be assayed and pellet them by centrifugation.
Note: The basic experiment includes both the experimental cell sample (i.e., those cells for which the lead or cadmium status is unknown) and the comparative control cell sample (i.e., cells that will be subjected to lead or cadmium at known concentrations).

2.2 Remove supernatant and resuspend each sample pellet in warm saline to wash cells.

2.3 Collect the cells by centrifugation and resuspend the cell pellet (s) in warm saline at 1 x 10^6 cells per mL.

2.4 Set up the following tubes, each containing 1 mL cell suspension:

- **2.4a** A tube (or series of tubes) containing the experimental cells.
- **2.4b** A series of tubes containing the comparative control cells.
- **2.4c** Single-color compensation control: using comparative control cells, set up a single tube containing 5 µL of propidium iodide (PI, Component F) to serve as the PI-positive compensation sample.

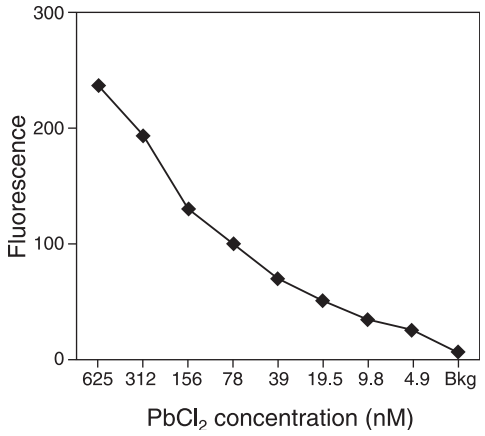

Figure 4. Approximate level of intracellular lead in experimental cells may be determined by comparing MFI values obtained to MFI values from the comparative control samples. Jurkat cells were loaded with Leadmium™ Green AM dye and washed. Samples were then incubated in the presence of $PbCl_2$ solutions in the range of 5–625 nM, and 1 µM ionomycin. After washing the samples, propidium iodide (PI) was added. Dual-color fluorescence was collected using 488 nm excitation and 525/10 nm and 610/10 nm bandpass filters. MFI measurements were recorded from a region on the dual-color plot drawn around the dye-positive and PI-negative population, and then plotted.

2.4e Single-color compensation control: using comparative control cells, set up a single tube containing 1 µM $PbCl_2$ in saline (or containing 100 µM $CdCl_2$ in saline) plus 2 µL ionomycin (Component D) to serve as the Leadmium™ Green dye–positive compensation sample.

2.4e Background: using comparative control cells, set up a single tube in saline with propidium iodide (PI) added (no exposure to lead or cadmium). This gives the background level of the Leadmium™ Green dye.

2.5 Add 4 µL of Leadmium™ Green AM dye working solution (prepared in step 1.3) to all tubes (except single-color PI compensation tube) and vortex briefly to mix.

2.6 Incubate tubes in a water bath at 37°C for 30 minutes, protected from light. This incubation time may be extended for up to 60 minutes, if desired.

2.7 Add 4 mL of warm saline to each tube.

2.8 Pellet cells by centrifugation.

2.9 Set up the tubes for second incubation as follows:

2.9a For experimental samples, add 1 mL warm saline to each tube.

2.9b For comparative control samples, add 1 mL of each concentration of $PbCl_2$ or $CdCl_2$ (see steps 1.5 and 1.6) and 2 µL 500 µM ionomycin (Component D) to this series of tubes.

2.9c For single-color PI compensation tube, add 1 mL warm saline.

2.9d For single-color Leadmium™ Green dye compensation tube add 1 mL either 1 mM $PbCl_2$ or 1 µM $CdCl_2$ and 2 µL ionomycin (Component D).

2.9e For dye background tube, add 1 mL saline.

2.10 Vortex all tubes briefly and incubate in a 37°C water bath for 30 minutes, protected from light.

2.11 Add 4 mL of warm saline to each tube.

2.12 Pellet cells by centrifugation.

2.13 Add 0.5 mL saline to all tubes and mix well.

2.14 Add 5 µL of 150 µM PI (Component F) to all tubes (except single-color Leadmium™ Green dye compensation tube) and vortex briefly to mix.

2.15 Incubate tubes for 5 minutes at room temperature, protected from light.

2.16 Put tubes on ice and protect from light. Analyze on the cytometer within one hour.

Flow Cytometric Analysis

3.1 Set the instrument for 488 nm excitation and use appropriate bandpass filters. A dual-parameter fluorescence plot with a region around dye-positive and PI-negative events may be used to gate out dead cells.

3.2 Run unstained cells to get main population of cells on the FSC/SSC plot and adjust the voltages of the fluorescence plots to put unstained cells in the first decade.

3.3 Run single-color PI compensation tube and adjust settings.

3.4 Run single-color Leadmium™ Green dye compensation tube and adjust settings.

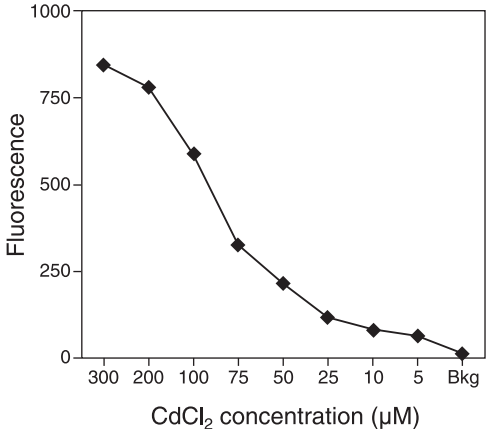

Figure 5. Approximate level of intracellular cadmium in experimental cells may be determined by comparing MFI values obtained to MFI values from the comparative control samples. Jurkat cells were loaded with Leadmium™ Green AM dye and washed. Samples were then incubated in the presence of $CdCl_2$ solutions in the range of 5–300 µM, and 1 µM ionomycin. After washing the samples, propidium iodide (PI) was added. Dual-color fluorescence was collected using 488 nm excitation and 525/10 nm and 610/10 nm bandpass filters. MFI measurements were recorded from a region on the dual-color plot drawn around the Leadmium™ Green dye–positive and PI-negative population, and then plotted.

3.5 Run dye background tube. Single-color Leadmium™ Green dye fluorescence will give data similar to that in Figure 1. Analyze dual-color fluorescence, Leadmium™ Green dye vs. PI. Exclude dead PI-positive cells by making a region around Leadmium™ Green dye–positive and PI-negative cell population.

3.6 Analyze remaining tubes. Collect and record MFI of the dye-positive and PI-negative region in the dual plot. Single-color fluorescence histogram of the intracellular lead is shown in Figure 2. Dual-color fluorescence of intracellular lead in live cells vs. PI-positive dead cells is shown in Figure 3. Graphs of the fluorescence values obtained when Leadmium™ Green–loaded Jurkat cells were treated with various concentrations of either $PbCl_2$ or $CdCl_2$ are shown in Figures 4 and 5 respectively.

3.7 To confirm that the observed fluorescence is due to the presence of lead or cadmium, it is possible to chelate the heavy metals. To do this, allow the tubes come to room temperature, add 2.5 µL TPEN, and vortex briefly to mix.

3.8 Incubate 5 minutes at room temperature.

3.9 Analyze the tubes at the same settings used for testing.
Note: TPEN is a cell-permeant, heavy metal chelator. Samples treated with TPEN should show a drop in fluorescence.

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

Cat #	Product Name	Unit Size
I36352	Intracellular Lead and Cadmium Detection Kit (Leadmium™ Green AM Detection) *for flow cytometry* *200 assays*	1 kit

Contact Information

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