EarlyTox Live Cell Assay Kit

The EarlyTox™ Live Cell Assay Kit enables the detection of the live population of mammalian cells based on the measurement of intracellular esterase activity using a fluorescence microplate reader.

Table 1-1: Available Kits

Assay Kit	Explorer Kit	Bulk Kit
EarlyTox Live Cell Assay Kit	R8342	R8343

Contents

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Chapter 1: About the EarlyTox Live Cell Assay Kit

The EarlyTox Live Cell Assay Kit provides a reagent that stains live cells with green fluorescence based on the intracellular esterase activity. The key features of this kit are the following:

- Optimized for fluorescence microplate readers, such as SpectraMax® readers
- Simple workflow – direct measurement in wells with or without medium removal
- Increased sample throughput with microplate format
- Preconfigured protocol in SoftMax® Pro Software

Assay Principles

Calcein AM is a widely used live-cell marker. The non-fluorescent calcein AM permeates the intact cell membrane and is converted into calcein, the fluorescent form, by intracellular esterases. The number of live cells are therefore indicated by the intensity of green fluorescence in the cytosol, with excitation at 495 nm and emission at 530 nm. Calcein AM has been used for studies of cell membrane integrity[1] and because of its lack of cellular toxicity, for long-term cell tracing as well.[2,3] It has also been used for quantifying viable cell numbers[2,3,4]. The EarlyTox Live Cell Assay Kit is an end-point assay for cell viability that is designed for use with fluorescence microplate readers.

Chapter 2: Materials and Equipment

Kit Components

Table 2-1: Components of the EarlyTox Live Cell Assay Kits

Item	Explorer Kit (R8342)	Bulk Kit (R8343)
Calcein AM, 4 mM in DMSO	1 x 30 µL	3 x 50 µL

- The Explorer kit is sufficient for two 96-well microplates.
- The Bulk kit is sufficient for ten 96-well microplates.

The number of microplates is based on the example protocol that is detailed in this document.

Storage and Handling

Reagents in this kit should be stored sealed, desiccated, protected from light, and frozen at –20°C. Allow the reagents to warm up to room temperature before opening. Before refreezing, seal all stock solutions tightly. Calcein AM is susceptible to hydrolysis when exposed to moisture. If the color of Calcein AM stock solution turns orange, discard the tube. When stored as directed, the kit is stable for at least 6 months from the date it is received.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: www.moleculardevices.com/support.

Materials Required but Not Provided

Table 2-2: Reagents and Supplies

Item	Suggested Vendor
Black-walled, clear bottomed microplates (96-well)	Corning 3904 or equivalent
Phosphate-Buffered Saline (PBS)	Corning 21-030 or equivalent

Compatible Molecular Devices Microplate Readers

- SpectraMax® i3x Multi-Mode Detection Platform
- SpectraMax® M2 and M2e Multi-Mode Microplate Readers
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M5 and M5e Multi-Mode Microplate Readers
- SpectraMax® Paradigm® Multi-Mode Detection Platform
- FlexStation® 3 Multi-Mode Microplate Reader
- Gemini™ EM and XPS Fluorescence Microplate Readers
- FilterMax™ F3 and F5 Multi-Mode Microplate Readers

Chapter 3: Assay Protocols

Example of Assay Protocol

The following protocol was used for an end-point assay of HeLa cells in the 96-well format. The optimal assay conditions for different cell types and plate formats can vary. Molecular Devices recommends testing a range of dye concentrations (1 to 10 µM), incubation times, and temperatures (room temperature or 37°C) to determine the optimal conditions for your assay system.

Working Solution Preparation

Use the following procedure to prepare a 2x working solution that is approximately 6 µM calcein AM:

1. Remove the calcein AM stock solution from the freezer and allow it to warm to room temperature.
2. Add 15 µL of the 4 mM calcein AM stock solution to 10 mL of PBS, and then vortex to ensure thorough mixing.

Note: 10 mL of this working solution is sufficient for one 96-well microplate.

CAUTION: Because aqueous solutions of calcein AM are susceptible to hydrolysis, use aqueous working solutions within one day. See Storage and Handling on page 4.

Assay Setup

1. Plate 20000 cells in 100 µL culture medium per well in a black-walled, clear-bottomed, 96-well microplate. Incubate in a 37°C, 5% CO_2 incubator overnight. If needed, include wells without cells as a background control.
   Molecular Devices recommends seeding cells at sufficient density to form a confluent.
   - For suspension cells, the recommended cell density is 40000 to 200000 cells per well.
   - For adherent cells, the recommended cell density is 20000 to 80000 cells per well.
2. Treat cells using the methods of your choosing for cell viability assays. Remember to include untreated wells as controls.
3. Add 100 µL of the calcein AM working solution to each well, which results in a final volume of 200 µL per well and a final concentration of 3 µM calcein AM.
4. Incubate the samples at room temperature for 30 to 60 minutes (or longer), protected from light.
5. Measure fluorescence in a fluorescence microplate reader with excitation at 495 nm and emission at 530 nm.

Fluorescence Microplate Reader Setup with SoftMax Pro Software

Table 3-1 displays typical fluorescence microplate reader settings. In SoftMax® Pro Software, use the preconfigured EarlyTox Live Cell Assay protocol that is available in the protocol library.

Table 3-1: EarlyTox Live Cell Assay protocol

Parameter	Setting
Read Mode	Fluorescence
Read Type	Endpoint
Wavelengths	Excitation = 495 nm, Emission = 530 nm
PMT and Optics	PMT Gain: Automatic Flashes per read: 6 Read From Bottom*

*Bottom read is preferred for cell-based assays, but top read can be used with microplate readers that do not have the bottom read option.

Chapter 4: Data Analysis Examples

Example Results

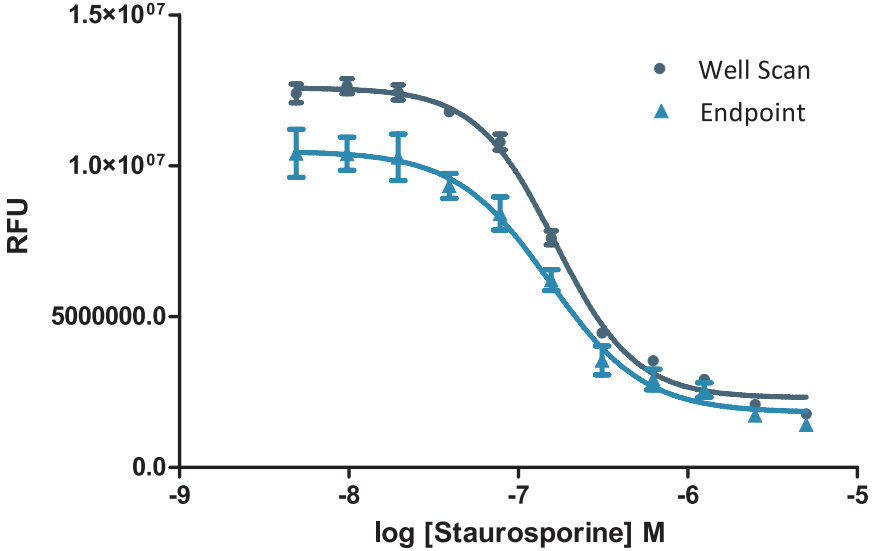

Figure 4-1: Cell viability assay: HeLa cells treated with staurosporine

HeLa at 20000 cells/well were plated in a 96-well microplate plate and grown overnight. Cells were then treated with staurosporine for approximately 24 hours at 37°C. 100 µL of the working solution of 6 µM Calcein AM (2x concentrations) was added directly to each well (n = 4). The samples were incubated at room temperature for about 60 minutes. The same assay plate was measured on a SpectraMax i3 Reader using Endpoint Read (Read from bottom) or Well Scan (Pattern: Fill; Points Per Well: 9; Density: 3; Point Spacing of approximately 1.51).

Assay Optimization Tips

- The example protocol was developed using HeLa cells, and can be adapted for use with other cell types. Assay signals can be optimized by adjusting the cell number and concentrations of the two dyes, generally between 1 and 10 µM.
- The example protocol described is a homogeneous protocol that does not replace the cell medium or use a wash step to simplify workflow and avoid disturbing cells. If needed, cell medium can be replaced with PBS prior to the addition of reagents to reduce background fluorescence. These manipulations, however, might result in inconsistent well-to-well signals because of disturbance or loss of cells.
- If available, try measuring the signals with both top and bottom read to determine the best instrument settings.
- If available, the Well Scan read type might improve the data quality and consistency.

Chapter 5: Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Please have the product name, part number, and lot number available when you call.

EarlyTox Product Family

Table 5-1: EarlyTox Product Family: Available Kits

Assay Kit	Explorer Kit	Bulk Kit
EarlyTox Live/Dead Assay Kit	R8340	R8341
EarlyTox Live Cell Assay Kit	R8342	R8343
EarlyTox Glutathione Assay Kit	R8344	R8345
EarlyTox Caspase-3/7 R110 Assay Kit	R8346	R8347
EarlyTox Caspase-3/7-D NucView 488 Assay Kit (DMSO Formulation)	R8348	R8349
EarlyTox Caspase-3/7 NucView 488 Assay Kit (PBS Formulation)	R8350	R8351

References

1. Wang XM, Terasaki PI, Rankin GW Jr, Chia D, Zhong HP, Hardy S. "A new microcellular cytotoxicity test based on calcein AM release." *Hum Immunol* **37**, 264 (1993).
2. Suuronen EJ, McLaughlin CR, Stys PK, Nakamura M, Munger R, Griffith M. "Functional Innervation in Tissue Engineered Models for InVitro Study and Testing Purposes." *Toxicological Sciences* **82**, 525 (2004).
3. Papadopoulos NG, Dedoussis GV, Spanakos G, Gritzapis AD, Baxevanis CN, Papamichail M. "An improved fluorescence assay for the determination of lymphocyte-mediated cytotoxicity using flow cytometry." *J Immunol Methods* **177**, 101 (1994).
4. De Clerck LS, Bridts CH, Mertens AM, Moens MM, Stevens WJ. "Use of fluorescent dyes in the determination of adherence of human leucocytes to endothelial cells and the effect of fluorochromes on cellular function." *J Immunol Methods*. 1994 Jun 3;**172**(1):115-24.

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