EarlyTox Glutathione Assay Kit

The EarlyTox™ Glutathione Assay Kit enables the detection of an early apoptosis event based on the measurement of the glutathione content in live mammalian cells using a fluorescence microplate reader.

Table 1-1: Available Kits

Assay Kit	Explorer Kit	Bulk Kit
EarlyTox Glutathione Assay Kit	R8344	R8345

Contents

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Molecular Devices, LLC is ISO 9001 registered.

Chapter 1: About the EarlyTox Glutathione Assay Kit

The EarlyTox Glutathione Assay Kit provides a convenient tool for assaying an early apoptosis event by measuring the glutathione content in live mammalian cells. 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

Apoptosis is a highly regulated and controlled process that leads to characteristic cellular changes, such as blebbing, cell shrinkage, chromatin condensation, chromosomal DNA fragmentation, apoptotic body formation[1], and eventually cell death. Diminished cellular glutathione (GSH), which is an important anti-oxidant in mammalian cells, occurs at the early stages of mitochondria-associated apoptosis because of GSH efflux.[2,3]

The EarlyTox™ Glutathione Assay Kit uses monochlorobimane (MCB), a cell permeant dye that has a high affinity for GSH, to detect the cellular GSH level in an apoptotic event.[3,4,5] The unreacted dye is almost non-fluorescent. After reaction of the dye with GSH, catalyzed by endogenous glutathione-S-transferase (GST) enzymes, the dye fluoresces blue, with excitation at 394 nm and emission at 490 nm. See Figure 1-1. The intensity of the fluorescence signal that is generated from the assay reflects the amount of GSH that is present in the cells.

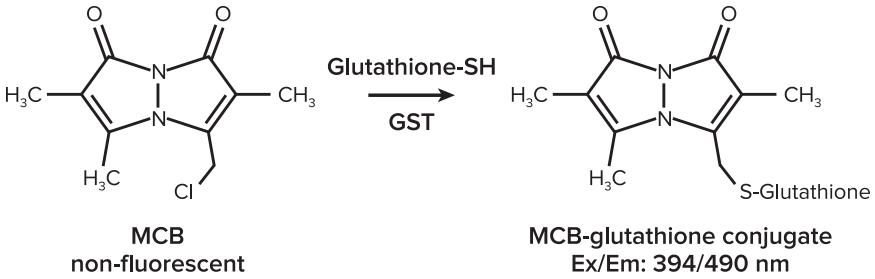

Figure 1-1: MCB glutathione assay principle

Chapter 2: Materials and Equipment

Kit Components

Table 2-1: Components of the EarlyTox Glutathione Assay Kits

Item	Explorer Kit (R8344)	Bulk Kit (R8345)
10 mM MCB	1 x 100 µL	1 x 500 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

Store at –20°C. 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 was used for an apoptosis assay of HeLa cells in the 96-well format. The optimal assay conditions for different cell types and microplate formats can vary. Molecular Devices recommends testing a range of MCB concentrations, incubation times, and temperatures (room temperature or 37°C) to determine the optimal conditions for your assay system.

Assay Setup

Molecular Devices recommends that you perform the following controls:

- Negative control: Cells not induced to undergo apoptosis.
- Positive control: Cells induced to undergo apoptosis.

Use the following procedure to set up the assay:

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. Molecular Devices recommends seeding cells at sufficient density to form a confluent monolayer in each well.
   - For suspension cells, the recommended cell density is 40,000 to 200,000 cells per well.
   - For adherent cells, the recommended cell density is 20,000 to 80,000 cells per well.
2. Induce apoptosis in cells using methods of your choosing. Remember to include untreated wells as controls.
3. Dilute 40 µL of 10 mM MCB stock solution in 10 mL of PBS for each 96-well microplate to make a 40 µM MCB working solution (2x concentration).
4. Remove cell medium and replace it with 100 µL of PBS/well.
5. Add 100 µL of MCB working solution into each well to make a final concentration of 20 µM MCB in the wells.
6. Incubate the cells at 37°C for 30 minutes to 3 hours, protected from light.
7. Measure fluorescence in a fluorescence microplate reader with excitation at 394 nm and emission at 490 nm.

Note: Fluorescence intensity might continue to change over time. Multiple readings might be necessary before a final assay protocol is established.

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 Glutathione Assay protocol that is available in the protocol library.

Table 3-1: EarlyTox Glutathione Assay protocol

Parameter	Setting
Read Mode	Fluorescence
Read Type	Endpoint
Wavelengths	Excitation: 394 nm Emission: 490 nm
PMT and Optics	PMT Gain: Automatic Flashes per read:20 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

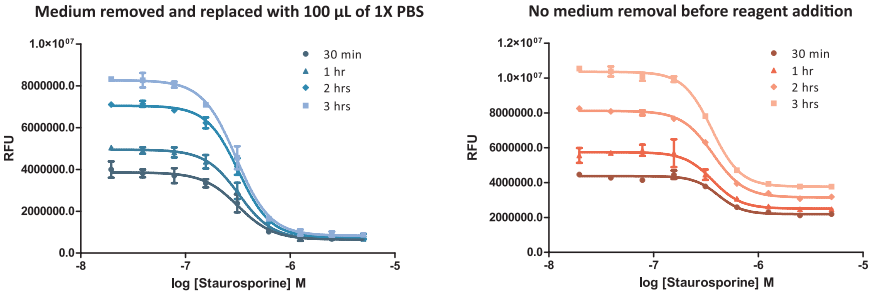

Figure 4-1: Apoptosis assay: HeLa cells treated with staurosporine

HeLa cells plated in a 96-well microplate overnight were treated with staurosporine for 5 hours at 37°C to induce apoptosis. Cell medium was replaced with 100 µL PBS (left) or not replaced (right) before 100 µL of 40 µM MCB (2x concentration) was added to each well. The samples were incubated at 37°C. Fluorescence was measured at 30 minutes, 1 hour, 2 hours, and 3 hours time points on a SpectraMax i3x microplate reader with excitation = 394 nm and emission = 490 nm, and read from the bottom.

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 MCB, generally between 10 µM and 100 µM.
- Incubating with MCB at 37°C generates higher signals than at room temperature. The MCB signal increases over time with improved signal to background ratio.
- The protocol can be further simplified by adding MCB reagent directly in the culture medium. This avoids disturbing cells because of washing or replacing the medium before running the assay. The overall signal to background ratio, however, is smaller, typically as the result of background fluorescence from the medium.
- Try measuring the signals with both top and bottom read to determine the best instrument settings.
- 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

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2. Coppola S, Ghibelli L. "GSH extrusion and the mitochondrial pathway of apoptotic signaling." *Biochem Soc Trans* **28**, 56 (2000).
3. Barhoumi R, Bailey RH, and Burghardt RC. "Kinetic analysis of glutathione in anchored cells with monochlorobimane." *Cytometry* **19**, 226 (1995).
4. Shrieve DC, Bump EA, Rice GC. "Heterogeneity of cellular glutathione among cells derived from a murine fibrosarcoma or a human renal cell carcinoma detected by flow cytometric analysis." *J Biol Chem* **263**, 14107 (1988).
5. Rice GC, Bump EA, Shrieve DC, Lee W, Kovacs M. "Quantitative analysis of cellular glutathione by flow cytometry utilizing monochlorobimane: some applications to radiation and drug resistance in vitro and in vivo." *Cancer Res* **46**, 6105 (1986).

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