APPLICATION NOTE

Wells to Westerns: Investigating the cellular heat shock response

Introduction

Investigating a cellular response can involve multiple approaches to gathering information—imaging, cell-based viability and proliferation assays, western blots to look at changes in protein expression, and more. Often multiple instrument platforms are needed to glean the necessary results, and several software packages may need to be learned in the process.

In this application note we show how data for several related cellular parameters was collected using a single instrument, the SpectraMax® i3 Multi-Mode Detection Platform. Heat shock was used as a model system to show how different detection modes, including imaging and Western blot scanning, can be used to gain insights into a multi-faceted cellular response.

Exposure of cells to higher than normal temperatures is known to activate the apoptosis pathway, as well as increase expression of the heat shock protein HSP70. CHO-K1 cells under two different growth conditions, healthy or stressed, were exposed to heat shock then assayed for proliferation, viability, and apoptosis using the imaging capability of the SpectraMax i3 system. Expression of HSP70, which is upregulated in response to heat shock, was analyzed using the ScanLater™ Western Blot Detection System.

Materials

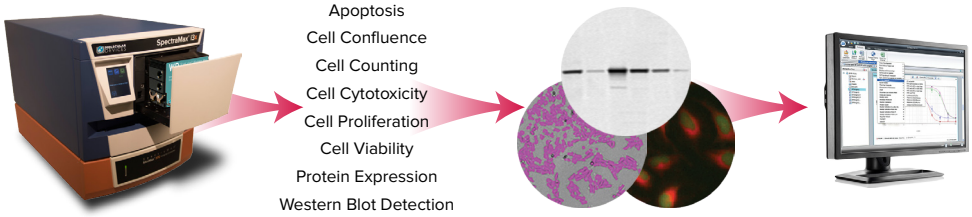
- CHO-K1 cells (ATCC P/N CCL-61)
- Cell culture medium (Ham's F12 with 10% fetal bovine serum and 1% penicillin/streptomycin)
- Black-wall, clear-bottom tissue culture microplates (Corning P/N 3603)
- SpectraMax i3 Microplate Reader
- SpectraMax MiniMax™ 300 Cytometer
- EarlyTox Cell Integrity Kit (Molecular Devices P/N R8213)
- CellEvent Caspase-3/7 Green Detection Reagent (Life Technologies P/N C10423)
- ScanLater Western Blot Detection System
  - ScanLater Anti-Mouse Evaluation Kit with Europium-labeled goat anti-mouse antibody (Molecular Devices P/N R8201)
  - ScanLater Western Blot Detection Cartridge
- Anti-HSP70 mouse monoclonal antibody (R&D Systems P/N MAB1663)
- Immobilon-FL Membrane, 0.45 µm pore size (EMD Millipore P/N IPFL 000 10)

Methods

Two populations of CHO-K1 cells were prepared, healthy and stressed. The healthy cells were given regular changes of culture medium and passaged at ≤ 80% confluence, while the stressed cells were allowed to become over-confluent and had less frequent replacement of their medium.

Benefits

- **Monitor cell proliferation without dyes**
- **Investigate mechanisms of cell death with fluorescent cell imaging**
- **Image cells, run cell-based assays, and detect western blots, all on one platform**



One complete solution. With available options such as the SpectraMax® MiniMax™ 300 Imaging Cytometer, ScanLater™ Western Blot cartridge, optimized reagents, and the industry-leading data acquisition and analysis tool SoftMax Pro, the SpectraMax i3x* Detection Platform allows you to explore cellular pathways and protein activation and expression in one system.

The day before heat shock, healthy or stressed cells were seeded at 7500 cells per well in 96-well black-wall, clear-bottom tissue culture microplates for imaging and cell-based assays. For Western blot samples, cells were seeded at 225,000 cells per well in 6-well tissue culture plates.

The day after seeding, half of the cell plates were heat shocked by exposure to 45°C for 90 minutes. Immediately after heat shock, cells were imaged with the transmitted light (TL) channel of the SpectraMax MiniMax 300 imaging cytometer to look for morphological changes. At 6 hours and 24 hours after heat shock, cells were imaged again to measure proliferation and re-examine morphology. StainFree™ Technology was used to count individual cells in each well without dyes.

Cell-based assays for apoptosis and cell viability were performed to discover whether heat shock had induced apoptotic or other cell death pathways, and whether the responses differed in the healthy and stressed cell populations. The CellEvent assay was used to quantify apoptotic cells under control and heat shock conditions. Molecular Devices® EarlyTox™ Cell Integrity Assay Kit was used to distinguish and count live and dead cells. Both assays were performed 6 and 24 hours after the heat shock period.

For Western blot, cells grown and treated in 6-well plates were trypsinized, pelleted, washed and lysed in PBS with 0.5% Tween and protease inhibitors. Lysates were centrifuged, and the supernatants were assayed for protein content. 2.1 µg of cell extracts were loaded on a 4-20% gel, transferred to a PVDF membrane, and probed with mouse anti-HSP70, followed by Europium-labeled anti-mouse secondary antibody. Protein bands were detected and quantified using the SpectraMax i3 reader with ScanLater Western Blot Detection Cartridge.

Cell proliferation

After heat shock, cells were allowed to recover for 6 or 24 hours prior to imaging and other assays. At the two time points, differences in cell number were quantified using StainFree cell

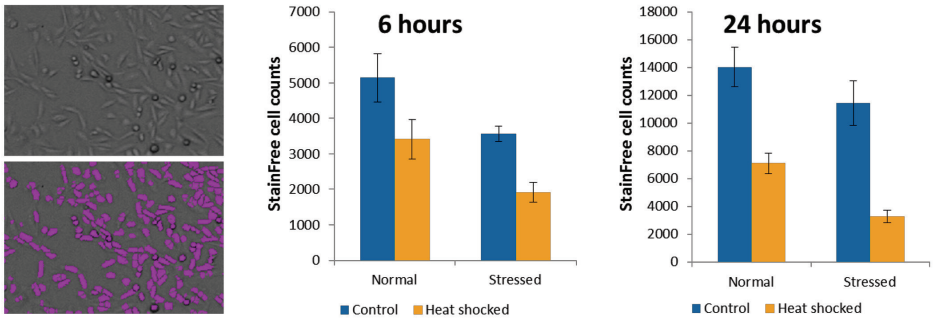


Figure 1. StainFree measurement of cell proliferation after heat shock. On the left are a transmitted light image of untreated CHO-K1 cells (top) and purple masks showing cells identified by StainFree analysis (bottom). Graphs show StainFree cell counts for normal and stressed populations of cells under control or heat shocked conditions. Cell counts were compared at 6 and 24 hours.

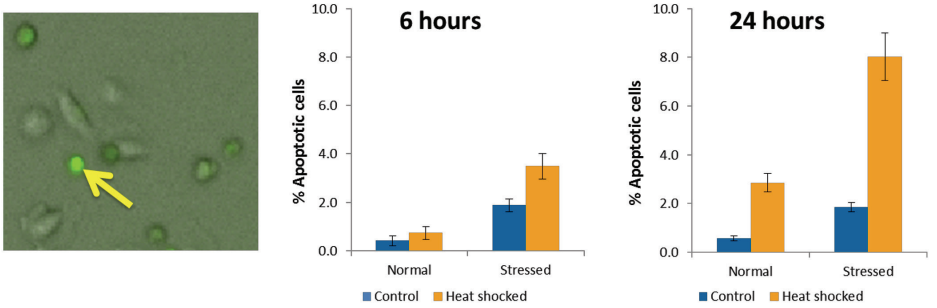


Figure 2. Apoptosis in control and heat-shocked cells. The left panel shows apoptotic (bright green) cells with rounded morphology, as well as several non-apoptotic cells cells with a more flattened, elongated shape. The yellow arrow points to an apoptotic cell with a high level of green fluorescence. The graphs show percent apoptosis in normal and stressed cell populations under control and heat shock conditions.

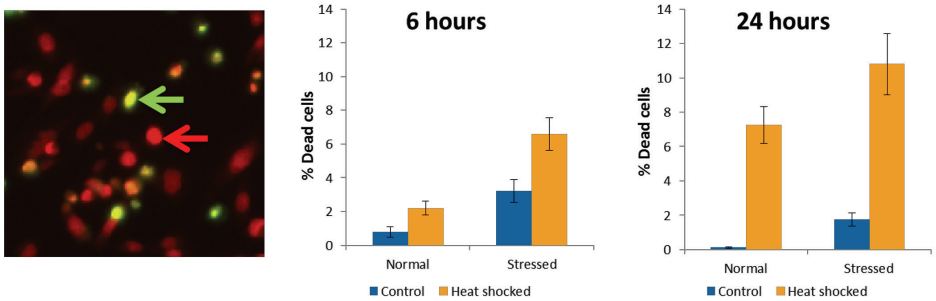


Figure 3. Quantitation of cell viability after heat shock. Heat shocked cells were imaged in the left panel (green arrow, dead cell; red arrow, live cell). Graphs show the increased percentage of dead cells upon heat shock, in both normal and stressed cell populations.

counting on the SpectraMax MiniMax 300 imaging cytometer. No dye was required for the software to identify and count cells. A predefined setting in the SoftMax® Pro Software was used to identify cells. A decrease in proliferation in heat-shocked cells, particularly in the stressed population, was observed, and it was more pronounced after 24 hours (Figure 1).

Apoptosis assay

At the 6 and 24 hour time points, cells were assayed for apoptosis. By 6 hours post-heat shock, stressed cells exhibited a higher percentage of apoptotic cells, but by 24 hours there were many more apoptotic cells in the stressed, heat-shocked population (Figure 2). By comparison, the non-heat-shocked cells maintained a steady and low percentage of apoptotic cells.

Cell viability assay

The EarlyTox Cell Integrity Kit was used to investigate whether cells were dying by other means than apoptosis. Increased cell death was observed with heat shock, particularly after 24 hours. Stressed cells had a higher overall percentage of dead cells than did the normal population. These results match reasonably well with the apoptosis result, suggesting that all or most of the cell death observed is due to apoptosis.

HSP70 induction

ScanLater Western blot analysis revealed that HSP70 was induced in response to heat shock as expected, but this response was much greater in the normal cell population than in stressed cells. With ScanLater analysis in SoftMax Pro Software, the intensity of bands on the blot was quantitated. A 48-fold increase in HSP70 was seen in the healthy cells 6 hours after heat shock, but only a 1.5-fold increase was seen in stressed cells (Figure 4). The amount of HSP70 expressed in non-heat-shocked control cells was about 18-fold higher for the stressed cell population than for normal cells.

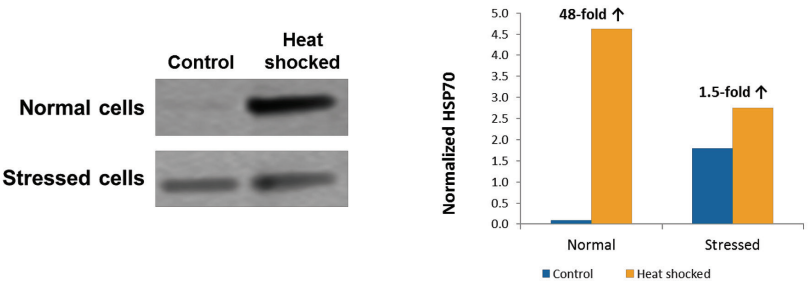


Figure 4. Western blot analysis of HSP70 expression. A Western blot for HSP70 expression in control and heat shocked cells, 6 hours after treatment, is shown in the left panel. Quantitation performed with ScanLater analysis in SoftMax Pro Software is shown in the graph. 'Normalized HSP70' indicates integrated density minus background, expressed in millions.

Conclusions

Using the SpectraMax i3 Multi-Mode Detection Platform, cell-based imaging assays and western blots were performed to examine the cellular response to heat shock. StainFree cell counting identified changes in proliferation with heat shock in normal and stressed cells. Apoptosis and cell viability assays provided insights into the mechanism of heat shock-induced cell death. Finally, Western blot analysis and enabled quantitation of the induction of HSP70 in control and heat shocked cells. From the results of apoptosis and cell viability assays, we hypothesize that all or most of the cell death occurring in response to heat shock was due to apoptosis. Induction of HSP70 was highest in the normal (non-stressed) cells under heat shock conditions. HSP70 was induced in stressed cells even prior to heat shock, and then induced further under heat shock. However, HSP70 in stressed cells never reached the high levels induced in healthy cells. As a result the stressed cells may have had less protection from apoptosis, leading to the higher percentage of apoptotic cells observed.

With the SpectraMax i3 system, and analysis enabled by SoftMax Pro Software, a multi-faceted investigation of cellular function that used to require multiple detection instruments and several different data analysis tools can now be performed on a single platform.

For more information on the SpectraMax i3x Multi-Mode Microplate Detection Platform, scan the QR code below or visit **moleculardevices.com/i3x**.

Watch the video, download the brochure, or view additional application notes.

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
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** Technical specifications for the SpectraMax i3x are similar to those of the SpectraMax i3. For luminescence, the LLD specification for the SpectraMax i3x is 5x lower (better) than for the SpectraMax i3.*