An Automated, High-Throughput Cell-Based Assay System using the Biomek 3000® Laboratory Automation Workstation for CellProbe™ HT Caspase-3/7 Whole Cell Assay

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Abstract

Using Beckman Coulter's CellProbe HT Caspase-3/7 Whole Cell Assay Kit in primary or secondary screening provides quality information on cellular responses to apoptotic regulators. This 35 µL total volume homogenous assay measures caspase-3/7 activities in a 384-well format. The high-throughput and low sample volume cell-based assay is achieved using the Biomek 3000 Laboratory Automation Workstation for cell preparation, apoptosis induction and substrate addition. The Affinity* Multi-Mode Plate Reader (Cambridge Research Institute, Boston) is an ultra-sensitive screening platform that uses a focused laser and CCD camera for fluorescence intensity detection. The caspase-3/7 activity is inducer-dose dependent and responder-cell number dependent. The dose response of DEVD-CHO inhibition of caspase-3/7 activities was used to demonstrate the superior sensitivity and specificity of this system. The Biomek 3000 Laboratory Automation Workstation facilitated CellProbe HT Caspase-3/7 Whole Cell Assay implementation, reduced the chance of contamination and minimized the intensive requirement of sterile skills. The automated CellProbe assay can be used for high-throughput screening of drug candidates for apoptosis regulators.

Introduction

Programmed cell death, apoptosis, is critical to the normal health and development of metazoic organisms. Several diseases have been linked to abnormalities in apoptosis. When apoptosis is inhibited, cancer and autoimmune diseases may occur and if apoptosis is overly active, stroke and Alzheimer's disease may result. To develop therapeutic agents for malfunctions of apoptosis, investigations have centered on the cascade of events that involve the proteins that regulate apoptosis. Among these proteins are a family of enzymes known as caspases, which are cysteine proteases that cleave substrates at aspartic acid residues. Caspases that act early in apoptosis are termed initiators and those that act late are effectors. Caspase-3 is an effector caspase that cleaves the amino acid sequence Asp-Glu-Val-Asp (DEVD).

This paper describes the CellProbe HT Caspase-3/7 Whole Cell Assay in 384-well format using the Beckman Coulter's Biomek 3000 Laboratory Automation Workstation and the Affinity Multi-Mode Plate Reader (Cambridge Research Institute, Boston). The Affinity reader is used for fluorescence intensity detection. It is an ultra-sensitive screening platform that uses a focused laser and CCD camera for detection. The Caspase-3/7 Whole Cell Assay, a 35 µL assay in 384-well format, detects intracellular caspase-3/7 activity with a whole cell approach, which has minimal background signals contributed by non-specific protease activities in a cell lysate assay; hence, higher specificity for caspase-3/7 activity can be achieved. The simple **"add, read, no mixing, no washing"** protocol provides easy and simple adaptation for high-throughput sample preparation using the Biomek 3000 Laboratory Automation Workstation. The addition of the Affinity Multi-Mode Plate Reader adds precision, speed and reliability to this high-throughput screening assay.

Materials and Methods

Automation Platform and Detection Instruments

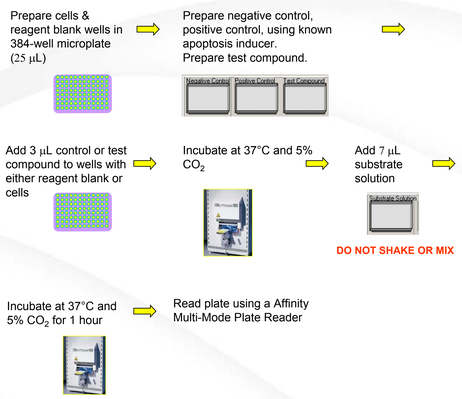
- Biomek 3000 Laboratory Automation Workstation in a biosafety hood (Baker Company, Maine)
- Affinity Multi-Mode Plate Reader (Cambridge Research Institute)

CellProbe HT Caspase 3/7 Whole Cell Assay

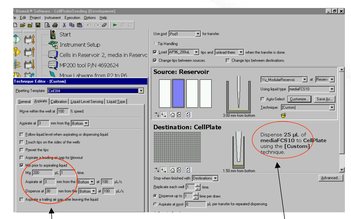
- Human cervical carcinoma HeLa cell line (ATCC)
- Human hepatoma HepG2 cell line (ATCC)
- Apoptosis inducers: staurosporin (STS), paclitaxel (Sigma-Aldrich Inc.)
- Caspase-3 inhibitor: Ac-DEVD-CHO (Molecular Probes Inc.)
- CellProbe HT Caspase-3/7 Whole Cell Assay kit (Beckman Coulter, Inc.)
- Fluorescence intensity : excitation at 498 nm and emission at 521 nm
- Biomek 3000 tools including the MP200 tool and the gripper tool

Biomek 3000 Laboratory Automation Workstation

CellProbe HT Caspase-3/7 Whole Cell Assay Procedure Summary



Biomek 3000 Software Screen Showing Parameters for Cell Seeding in Microplates



Mixing ensured even cell seeding in microplates.

25 µL of cells from a reservoir were dispensed into each well of the 384-well microplates.

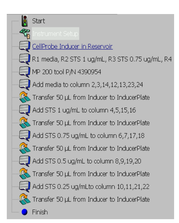
Biomek 3000 Software Screen Showing Parameters for Compound and Control Preparation

Control & Compound Preparation

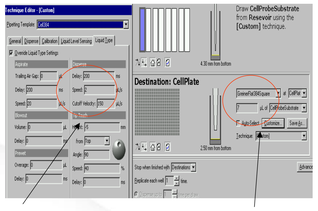
Master plate of compounds or negative/ positive controls were transferred to stock plates.

Control & Compound Addition

3 µL of stock plate reagent were added to each well of the cell plate.



Biomek 3000 Software Screen Showing Parameters for Substrate Addition

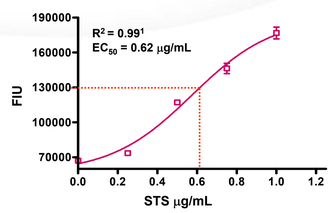


Slow dispense rate for substrate delivery to ensured maximum signal.

7 µL of substrate were added to each well of the microplate.

The Affinity Muti-Mode Plate Reader, an Ultra-Sensitive Screening Platform Uses a Focused Laser and CCD Camera for Detection

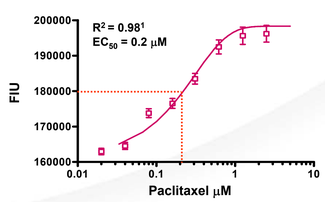
Figure 1: Staurosporin EC_{50} Dose Response using CellProbe HT Caspase-3/7 Whole Cell Assay in Detecting Apoptotic HeLa Activity



STS at different concentrations induced caspase-3/7 activity at 5 hours of stimulation.

[1] Second order non-linear regression analysis

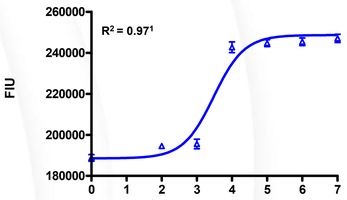
Figure 2: Paclitaxel EC_{50} Dose Response using CellProbe HT Caspase-3/7 Whole Cell Assay in Detecting Apoptotic HeLa Activity



Paclitaxel at different concentrations induced caspase-3/7 activity at 18 hours of stimulation.

[1] Second order non-linear regression analysis

Figure 3: Paclitaxel Dose Response using CellProbe HT Caspase-3/7 Whole Cell Assay in Detecting Apoptotic HepG2 Activity



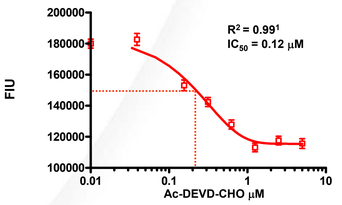
Paclitaxel at different concentrations induced caspase-3/7 activity at 18 hours of stimulation.

[1] Second order non-linear regression analysis

Table 1: Detection of Inducer EC_{50} Dose using CellProbe HT Caspase-3/7 Whole Cell Assay in HeLa and HepG2

	Staurosporin EC_{50} Dose 5 hour incubation	Paclitaxel EC_{50} Dose 18 hour incubation
HeLa	0.62 µg/mL	0.2 µM
HepG2		~3.5 µM

Figure 4: Specificity of the CellProbe HT Caspase-3/7 Whole Cell Assay in Detecting Apoptotic HeLa Activity



STS (1 µg/mL) induced apoptotic cells were treated with increasing concentrations of the inhibitor, Ac-DEVD-CHO. Ac-DEVD-CHO competed with the substrate for caspase-3/7 in apoptotic cells.

[1] Two phase exponential decay kinetic analysis

Conclusions

- CellProbe HT Caspase-3/7 Whole Cell Assay is a simple "**add, read, no mixing, no washing**" assay detecting intracellular caspase-3/7 activity in 384-well format.
- The assay detects intracellular caspase-3/7 activity with great sensitivity as demonstrated by STS and paclitaxel dose dependent activities, and EC_{50} dose.
- The assay detects intracellular caspase-3/7 activity with great specificity as demonstrated by Ac-DEVD-CHO inhibitor competition activities.
- The assay can be used to evaluate compounds inducing apoptosis and toxicity through the caspase-3 cascade.
- The assay can also be used to evaluate compounds that are interfering with caspase-3/7 activity in apoptotic cells.
- The automated whole cell assay system using the Biomek 3000 Laboratory Automation Workstation facilitated cell-based assay implementation by reducing the chances of contamination, and by precise delivery of low volume reagents.
- The automated CellProbe HT Caspase-3/7 Whole Cell Assay can be used as high-throughput screening of apoptotic regulators with great sensitivity and reproducibility.