Automation of a Cell-Based Screening Assay for Intracellular Apoptosis Activity using the Biomek® Assay Workstation

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Abstract

Assessment of drug-induced toxicity in tumor cells and hepatotoxicity in hepatocytes is an important aspect of new drug development. The CellProbe™ HT Caspase-3/7 Whole Cell Assay kit provides quality information on cellular responses to apoptotic regulators. This whole-cell screening assay measures caspase 3/7 activities in intact cells. Automation of the assay is achieved using the Biomek Assay Workstation with a Biomek NX integrated with a Cytomat* incubator and storage carousel for cell preparation, apoptosis induction and detection. This system was used to study the staurosporin dose-dependent induction of caspase 3/7 activities in both HepG2 and HeLa cells. The dose-dependent inhibition of caspase 3/7 activities was determined using DEVD-CHO. These hepatoma and tumor cellular models can be used to calculate the EC_{50} dose of apoptosis inducers and the IC_{50} dose of apoptosis inhibitors. This automated cell-based screening system, with superior sensitivity and specificity, can be used for evaluating pharmacological properties of 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 termed effectors. Caspase 3 is an effector caspase that cleaves the amino acid sequence Asp-Glu-Val-Asp (DEVD).

This poster describes the performance of the CellProbe HT Caspase-3/7 Whole Cell Assay on Beckman Coulter's Biomek Assay Workstation using a Biomek NX Multichannel (MC) platform integrated with a Cytomat CO_2 incubator, pipette tip storage carousel and the DTX 880 Multimode Detector, all running under the control of SAMI® Workstation EX 4.0 scheduling software. The system scheduled a six hour method to process ten assay plates from apoptosis induction to detection of caspase 3/7 activity without the need for operator intervention. The Caspase-3/7 Whole Cell Assay detects intracellular caspase 3/7 activity with a whole cell approach, which has minimal background signal contributed by non-specific protease activities; 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 Assay Workstation.

Materials and Methods

Automation Platform and Detection Instruments

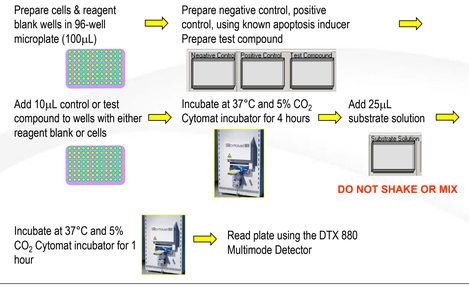
- Biomek Assay Workstation using a Biomek NX MC Automated Workstation integrated with:
  - Cytomat incubator at 37°C with 5% CO_2 and 100% humidity
  - Cytomat storage carousel for pipette tips
  - DTX 880 Multimode Detector
  - Control by SAMI Workstation EX 4.0 scheduling software

CellProbe HT Caspase-3/7 Whole Cell Assay

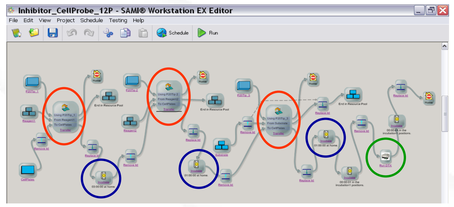
- Human cervical carcinoma HeLa cell line (ATCC)
- Human hepatoma HepG2 cell line (ATCC)
- Apoptosis inducers: staurosporin (STS) (Sigma-Aldrich Inc.)
- Caspase 3 inhibitor: Ac-DEVD-CHO (Biosciences, Inc.)
- CellProbe HT Caspase-3/7 Whole Cell Assay Kit (Beckman Coulter, Inc.)
- Fluorescence intensity: excitation at 498 nm and emission at 521 nm

Biomek Assay Workstation with Integrated Biomek NX MC, Cytomat Incubator, Storage Carousel, DTX 880 Multimode Detector

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

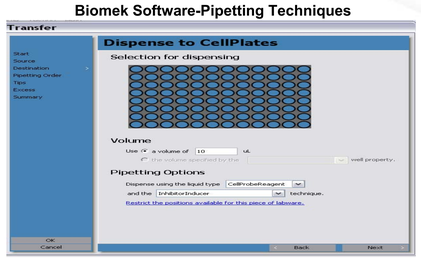


Biomek Assay Workstation SAMI Software Work Flow



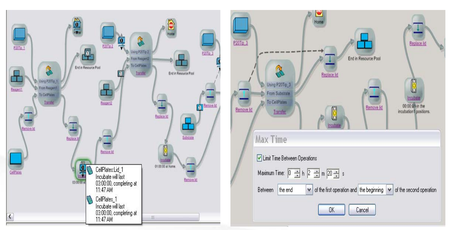
Transfer Steps Incubation Steps DTX 880 Multimode Detector Step

Biomek Assay Workstation SAMI Software Biomek Software-Pipetting Techniques



Liquid type and pipetting technique are controlled by the Biomek software techniques.

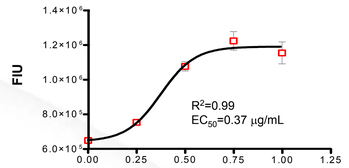
Biomek Assay Workstation SAMI Software Incubation Time and Max Time



End of incubation time for the scheduled method

Max Time allows restricted time limit between the two procedures.

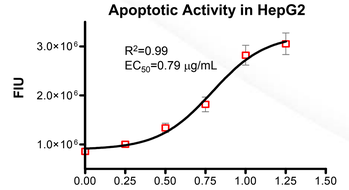
Staurosporin EC_{50} Dose Response in Detecting Apoptotic Activity in HeLa



Staurosporin at different concentrations induced caspase 3/7 activity at 4 hours of stimulation. Data shown as sigmoidal dose-response (variable slope):

$y = 0.64x10^6 + (1.19x10^6 - 0.64x10^6)/(1 + 10^{((0.37-x)*4.92)})$.

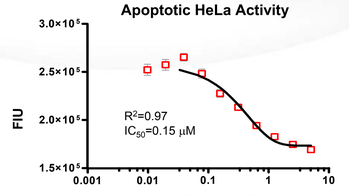
Staurosporin EC_{50} Dose Response in Detecting Apoptotic Activity in HepG2



Staurosporin at different concentrations induced caspase 3/7 activity at 4 hours of stimulation. Data shown as sigmoidal dose-response (variable slope):

$y = 0.89x10^6 + (3.24x10^6 - 0.89x10^6)/(1 + 10^{((0.79-x)*2.61)})$.

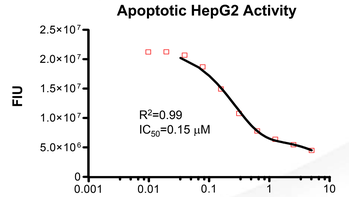
Staurosporin IC_{50} Dose Response in Detecting Apoptotic HeLa Activity



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. Data shown as two phase exponential decay kinetic analysis:

$y = 1.44x10^5*\exp(-0.017x) + 0.77x10^5\exp(-2.57x) - 0.37x10^5$.

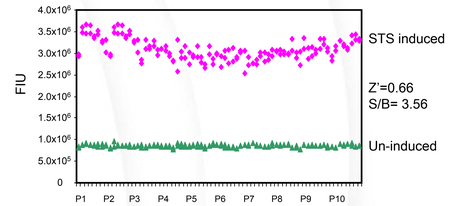
Staurosporin IC_{50} Dose Response in Detecting Apoptotic HepG2 Activity



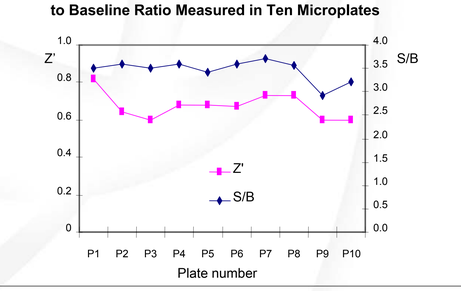
STS (1 µg/mL) induced apoptotic cells were treated with increasing concentrations of the inhibitor, Ac-DEVD-CHO. Ac-DEVD-CHO competed with the substrate for caspase 3/7 in apoptotic cells. Data shown as two phase exponential decay kinetic analysis:

$y = 1.02x10^{7}*\exp(-0.045x) + 1.55x10^{7}\exp(-3.73x) - 0.36x10^{7}$.

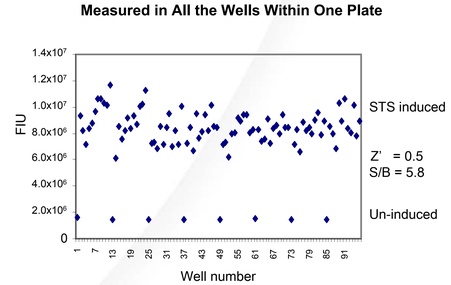
Staurosporin Induced Caspase 3/7 Activity Measured in Ten Microplates



Signal Stability including Z' Factor and Signal to Baseline Ratio Measured in Ten Microplates



Staurosporin Induced Caspase 3/7 Activity Measured in All the Wells Within One Plate



Conclusions

- The cell-based assay is automated using Beckman Coulter's Biomek Assay Workstation integrated with the Biomek NX MC platform, Cytomat CO_2 incubator, pipette tip storage carousel and DTX 880 Multimode Detector, all under the control of SAMI Workstation EX 4.0 scheduling software.
- The Biomek Assay Workstation SAMI Software scheduled a ten-microplate method in 6 hours for the CellProbe HT Caspase-3/7 Whole Cell Assay, without operator intervention.
- The assay detects intracellular caspase 3/7 activity with great sensitivity as demonstrated by STS 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 automated CellProbe HT Caspase-3/7 Whole Cell Assay can be used as high-throughput screening of apoptotic regulators with great sensitivity and reproducibility.