Automated System for Compound Library Screening by MTS Cell Viability Assays

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Abstract

Cell proliferation, cytotoxicity and other viability assays play an important role the drug discovery process. Many screening programs target anti-cancer compounds, requiring in-vitro characterization of efficacy. Additionally, all potential therapeutic compounds must be characterized as to cytotoxicity regardless of the target (ADME/Tox).

Cytokinetics has developed a system to automate the process of reagent addition, incubation and reading of cell viability assays utilizing the MTS method of Promega, Inc. The integrated system includes hardware from a variety of sources and software developed by Cytokinetics.

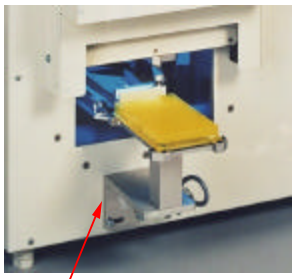
Control of environmental conditions including CO_2, temperature and humidity are important factors in accurate and reproducible cell-based assays. The system described here includes the Heraeus® Cytomat® 6000 series incubator from Kendro for incubation of cells.

Hardware

The system includes the Multimek™ from Beckman Coulter for reagent dispensing, the PlateCrane™ from Hudson Controls for plate handling, the BioRad Ultramark for reading absorbance and the Heraeus Cytomat® 6000 incubator for incubation.

Cytomat® 6000

The Heraeus Cytomat® 6000 from Kendro is a robot accessible CO_2 incubator. The incubator features internal plate handling, CO_2 and temperature control and humidification. The instrument is available in a variety of temperature ranges and configurations for easy integration into any automated system.



Cytomat Transfer Station offers a ***single point of access*** for all automation systems

Cytomat gate (Figure 1): the gate is open for a short time, and the small opening allows the system to maintain a stable environment (Figure 2)

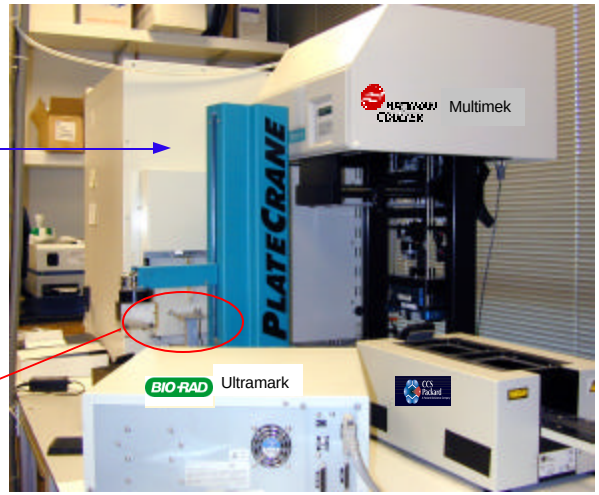
Multimek:

The Beckman Coulter Multimek™ is a 96-Channel, 6 position liquid dispensing system that can be used for 96 or 384 well dispensing.

PlateCrane:

The Hudson PlateCrane™ is a cylindrical robotic arm that delivers labware to one or more devices. This is the primary plate handling device for this system, moving plates between the Multimek, Ultramark, and Cytomat.

Figure 7



Software

Software drivers for the Cytomat, CCS Barcode reader, Hudson PlateCrane and Multimek were written at Cytokinetics using Visual Basic. Bio-Rad provided an OCX driver for integration of the plate reader.

Incubation times are tightly controlled (± 10 ms) using High Performance Timer Objects from Common Control Replacement Project (www.mvps.org/ccrp/) . Plate data is extracted, formatted and stored for automated import into Activity Base.. This is 10 times more efficient than HEPA filtration!

Assay and reagents

The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay is a colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. The CellTiter 96® AQueous Assay is composed of solutions of a novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium, inner salt; MTS and an electron coupling reagent (phenazine methosulfate) PMS. MTS is bioreduced by cells into a formazan that is soluble in tissue culture medium. The absorbance of the formazan at 490nm can be measured directly from 96 well assay plates without additional processing.

The conversion of MTS into the aqueous soluble formazan is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture.

The absorbance at 490 nm is read and recorded by the software.

Protocol

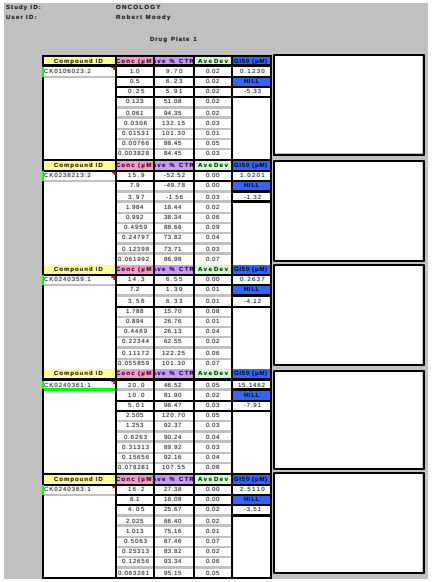
Cells are plated (50 µl) in 384 well plates and incubated overnight. Library compounds are added (50 µl) and the plates are returned to the incubator for a 48 hour incubation.

After this period, MTS is added and the plates are incubated for 2 (two) hours. Plates are then read at 490 nm and recorded by the software.

Promega

Results

All test compounds used to validate the assay protocol show dose responses (Table 1, Tables 2-5 and Figure 6) as expected and previously established using manual procedures.



Summary

The system described here allows automation of cell viability, cell proliferation or cytotoxicity assays in a 96 well format in a very small footprint. All hardware components are controlled with VB drivers (except the OCX driver for the Bio-Rad Ultramark) in Windows NT. Each driver took approximately 1 FTE week to complete and implement. This system provides a flexible workstation for cell-based assays with a footprint of about 1.7 x 1.0 meters and is easily reconfigurable due to the open architecture used for all devices.

The next steps for this system will be to write a scheduler to enhance flexibility and to automate the cell plating and culture procedures.