Automação para ensaios de Kinase usando base 3D

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Application Note

Cell-Based Assays (Cellular Kinase Assays)

Automation of HTRF® eIF4E Kinase Assay Using a 3D Tumoroid-Based Cell Model

Incorporation of the MultiFlo™ FX Microplate Dispenser to Create 3D Cell Cultures using the RAFT™ System and Perform the Steps of an HTRF® Cellular Kinase Assay

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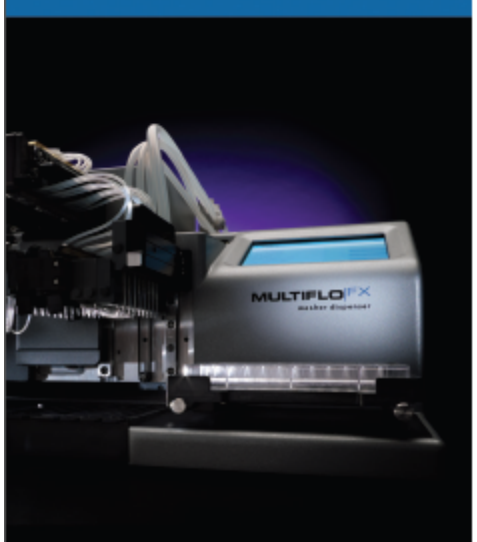


Key Words:

Microscopy
HTRF®
TR-FRET
Cell Signaling
eIF4E
3D
RAFT™
Tumoroid
Automation

Three-dimensional (3D) cell culture is poised to meet the need for a more *in vivo*-like cellular model with which to test large and small molecules. This is accomplished by providing a method that allows for the reorganization of cells into a format which re-establishes the necessary cellular architecture and communication networks seen in normal tissue. Recently a methodology has been put forth that incorporates a simplified procedure for the creation of a cell and collagen hydrogel mix. The inclusion of appropriate liquid handling instrumentation can further simplify the process and ensure the generation of accurate, robust results.

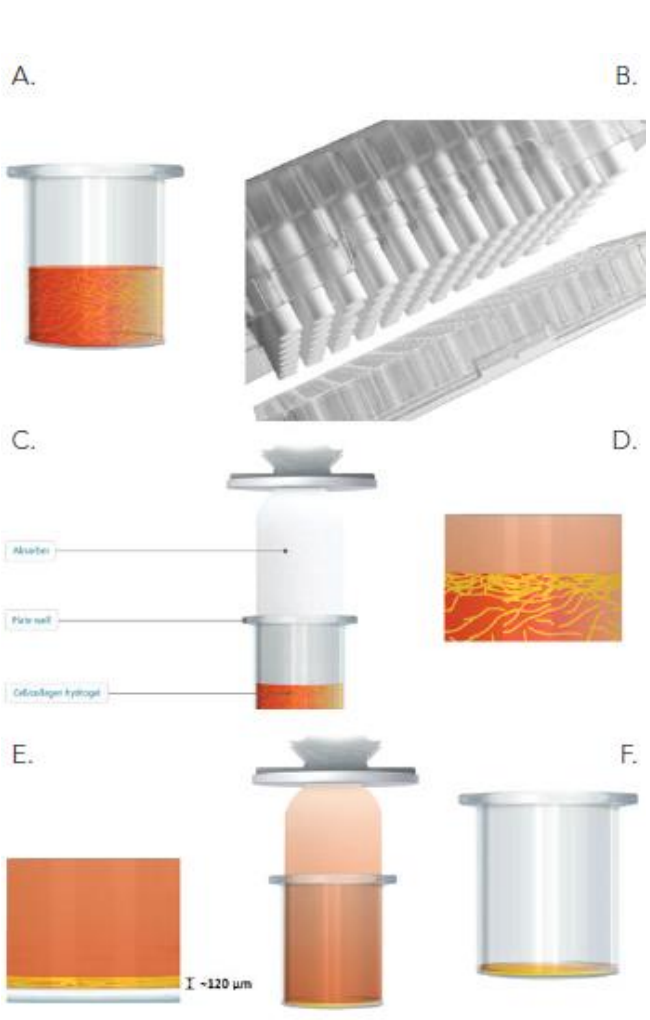
Introduction

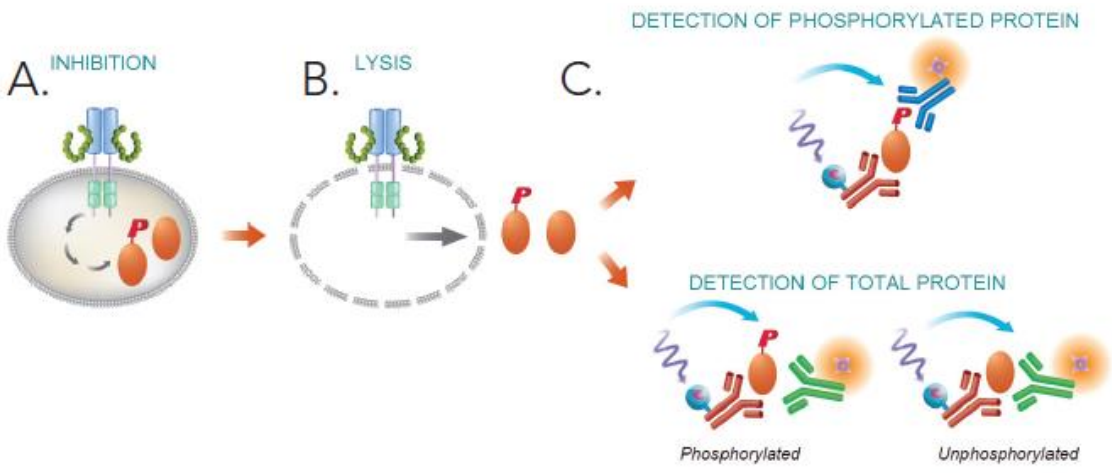
A central focus for improving drug efficacy in clinical trials over the last decade has been to increase the biological relevance of assays performed early in the drug discovery process. Biochemical assays used in screening campaigns are being replaced by cell-based, functional assays at ever increasing rates. Initially, these assays typically used the over-expression of drug targets in immortalized cell lines; but now more and more assays are conducted with human primary cells with endogenous expression of drug targets.

Yet it remains difficult to simulate an *in vivo* response to drug using an *in vitro* assay, where the cells are grown on hard plastic or glass substrates, in a two-dimensional (2D) format which is not representative of the *in vivo* cellular environment[1]. When examining cells within a tissue, it can be observed that cells interact with neighboring cells, and with the extracellular matrix (ECM) to form a communication network. This communication controls a number of cellular processes including proliferation, migration, and apoptosis[2]. However, most of the tissue-specific architecture, cell-cell communication, and cues are lost when cells are grown in a more simplified 2D manner. Therefore, more advanced cell culture methods are required to better mimic cellular function within living tissue.

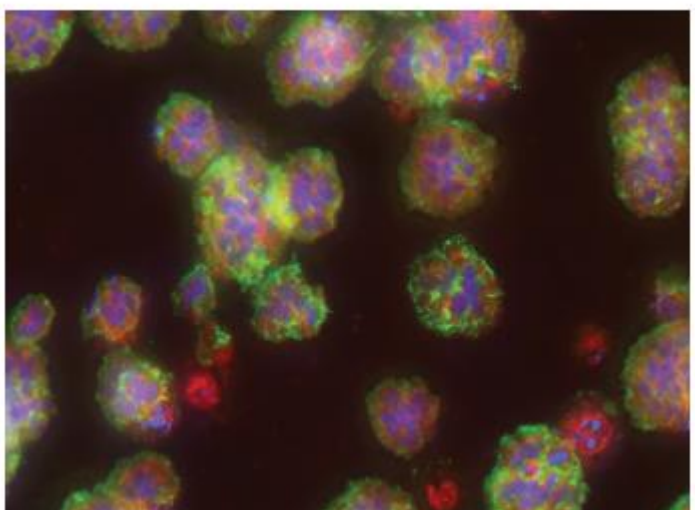
3D cell culture serves to meet this demand by providing a matrix that encourages cells to reorganize into a structure more indicative of an *in vivo* environment; thereby allowing normal cell-cell and cell-extracellular matrix (ECM) interactions to develop in an *in vitro* environment. However, as with any assay procedure performed in a cell-based manner, it is imperative that correct instrumentation be incorporated for dispensing and cell washing to further ensure that proper conclusions can be made with these promising cell models. The robustness of the 3D cell culture structure to automated aspiration and dispensing of media, compounds and reagents will be assessed.

In this application note we demonstrate an *in vitro* microplate assay that can quantify total, as well as phosphorylated eIF4E. The assay was performed wherein cells were cultured using a novel 3D culture system called RAFT™ to create a cell/collagen hydrogel mix. The assay workflow involved a two plate protocol where cells are plated and compounds are added to inhibit basal activity of the eIF4E signaling pathway. Levels of phosphorylated and total eIF4E are then quantified by transferring the cell lysates to a second plate and detection reagent addition. All dispensing and removal steps were performed by the MultiFlo™ FX Microplate Dispenser, including cell/collagen mix, medium, and reagent dispensing, as well as removal of spent medium and compounds. Validation data generated using the automated assay procedure confirms the ability of the instrument to perform accurate, repeatable addition and removal steps throughout the entire process.





Cytation3 Imaging of 3D Tumoroid Structures



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