ESSEN
INSTRUMENTS

IncuCyte™ Applications

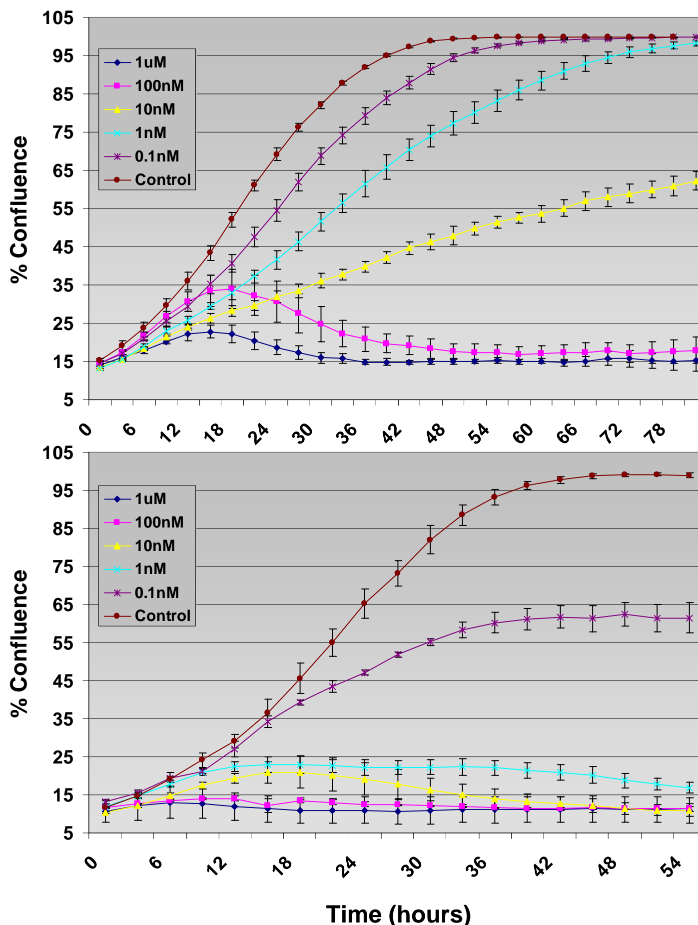
Proliferation Assays

Quantifying cellular proliferation is one of the most fundamental means for monitoring cells in culture. Proliferation assays are vital in pre-clinical drug discovery for specific therapeutic target areas, such as oncology, and for *in-vitro* cytotoxicity assays.

Many techniques have been developed to measure cellular proliferation including: quantifying DNA synthesis (^{3}H-Thymidine, *BrdU, DNA dye staining), intracellular reduction-oxidation (**MTT), and antigen detection (ELISA). All of these techniques can be performed in a microplate format and, as such, are extendable to high-throughput assays. However, these techniques suffer from several disadvantages. The IncuCyte™ provides a novel method for the quantification of cellular proliferation which eliminates many disadvantages associated with currently available assay protocols.

The strength of the IncuCyte™ platform lies in its ability to generate kinetic growth curves directly from phase contrast imagery. Furthermore, the kinetic images are generated inside your incubator, eliminating the need to remove the cells from their controlled environment. This non-invasive, label-free approach is cheaper, more precise, saves time, and bypasses the need for expensive robotic automation.

Titration of Antibiotic on CHO and HT-1080 Cells



Actinomycin-D CHO

Shown are the anti-proliferative effects of various concentrations of the antibiotic Actinomycin-D on CHO cells. Time updates were gathered every 3 hours, and the plot shows monolayer confluence vs. time.

Actinomycin-D HT-1080

Shown are the anti-proliferative effects of various concentrations of the antibiotic Actinomycin-D on HT-1080 cells (fibrosarcoma). Time updates were gathered every 3 hours, and the plot shows monolayer confluence vs. time.

Cellular proliferation depends on many variables including both seeding density and cell type. As such, seeding densities must be adjusted to generate consistent growth curves that, once established, are reproducible. IncuCyte™ can be used to establish and maintain optimized seeding parameters as well as to track consistency of the resulting proliferation curves.

The kinetic assay provided by IncuCyte™ can also help determine compound potency. Fixed "end-point" protocols can generate different results depending on the lag time required before the compound affects proliferation. A full-kinetic profile can help elucidate these discrepancies as well as the mechanism of action of the compound.

Use IncuCyte™ to:

- Elucidate time-dependent action of compounds
- Optimize media, substrate and growth conditions
- Optimize seeding densities
- Determine the optimum sampling window for an end-point HTS assay

Currently Available Techniques	IncuCyte™
1. Non-kinetic: Methods are incompatible with live cell-culture, sensitive to seeding density and assay read-out time.	**1.** IncuCyte™ generates full, kinetic growth curves which provide a precise, sensitive and more informative read-out.
2. Labeled: Quantified cells must be labeled with a with a marker, adding time, cost and possibly interfering with the assay detection method.	**2.** No label is required. Quantification derived from phase contrast imagery and cells are not altered in any way.
3. Invasive: Cells have been labeled; they can no longer be used for any subsequent experiments.	**3.** Non-invasive: Cells are not altered in any way during quantification; the exact same cells can be used for subsequent experiments.
4. Cells must be removed from the incubator for labeling or processing.	**4.** Cells never the leave the controlled incubator environment.
5. High throughput: HTS compatible	**5.** Not high throughput: Detailed analysis for assay optimization, target ID & validation, or lead optimization.

* 5-bromodeoxyuridine
** 3-(4,5-dimethyethiazol-2-yl)-2,5-diphenyltetrazolium bromide

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