

Understanding Proliferation and Differentiation Assays

WHAT IS PROLIFERATION AND DIFFERENTIATION?

- **Proliferation:** The expansion of cells by the continuous division of single cells into two identical daughter cells.
- **Differentiation:** The process whereby an undifferentiated cell acquires the features of a specialized cell.

RELATIONSHIP BETWEEN PROLIFERATION AND DIFFERENTIATION

- Proliferation occurs prior to differentiation.
- Without proliferation, differentiation would not occur.
- Differentiation is a default program requiring prior proliferation, but the corollary is not true.
- If proliferation is affected, then differentiation will also be affected, but not visa versa.

CHARACTERISTICS OF PROLIFERATION AND DIFFERENTIATION ASSAYS

- The processes of proliferation and differentiation overlap, but they are two separate processes.
- A proliferation assay does not measure differentiation and a differentiation assay does not measure proliferation.
- Measuring proliferation requires a different readout to that of differentiation.
- It is not possible to measure proliferation and differentiation using the same assay, but it is possible to combine different readouts in the same assay.
- In general, proliferation assays are quantitative since they are dependent upon the measurement of a biochemical reaction or interaction.
- Proliferation assays can be calibrated, standardized and validated.
- In general, differentiation assays are subjective, or semi-quantitative at best, and are therefore difficult, if not impossible, to calibrate, standardize and validate.

Examples of Proliferation Assays

Assay Type	Assay Readout
Isotope labelling	³ H-TdR, ⁵¹ Cr
Cell counts (e.g. Neutral red, methylene blue)	Manual / Automated
MTT, XTT, BudR, Edu	Absorbance
Calcein, CellQuant®	Fluorescence
Intracellular ATP	Bioluminescence

Examples of Differentiation Assays

Assay Type	Assay Readout
Molecular expression	PCR
Intracellular expression	Image analysis, ELISA, flow cytometry
Extracellular membrane expression	Image analysis, ELISA, flow cytometry
Colony-forming cell assay	Manual, image analysis

Choosing the correct assay for the study goals can reduce the risk of falsely interpreting the results.



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