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Evaluating Small Molecule Cell Cycle and Chromatin Condensation Modulators Using a Novel Green Fluorescent DNA-Binding Probe: Potential Application to Compound Screening

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ABSTRACT

When eukaryotic cells undergo replication, they pass through a tightly regulated series of events known as the cell cycle, marked by distinctive characteristics as the DNA is replicated. Cell cycle checkpoints at specific points in the process prevent cells from progressing to the subsequent phase of the cell cycle in the event of DNA damage or other adverse conditions that might impact overall survival. Considerable progress has been made in the analysis of the stages of cell cycle progression, with a number of cell permeable small molecules identified to modulate the process. Flow cytometry is a commonly implemented platform for cell cycle analysis, but the most widely employed dye for the instrument, propidium iodide, requires permeabilization or fixation of cells. A number of newer cell-permeable dyes have subsequently been introduced but most require very restrictive staining conditions and laborious standardization of techniques. We have introduced a novel fluorescent probe that facilitates cell cycle analysis in live cells. The green fluorescent probe can be used in a mix and read format over a wide concentration range (5-20 µM) employing a wide range of cell densities (1x 10^5-1x10^6 cells/ml). Moreover the dye provides substantial flexibility with respect to the incubation medium, time and temperature used in the analysis. Live cell cycle analysis was benchmarked using a panel of 12 small molecule cell cycle modulators known to perturb cells at the G_1/G_1, S or G_2/M phases in a concentration-dependent manner. Apoptotic cell death was monitored as well through protocol modifications allowing sub G_1 analysis and chromatin condensation determination. The described fluorescent probe should be applicable to the analysis of the phases of the cell cycle, especially as applied to the identification of small molecule modulators for use in treatment strategies targeting cell cycle checkpoints.

INTRODUCTION

The progression of the cell cycle is controlled by a complex interplay among various cell cycle regulators that either stimulate or inhibit the cell from entering each stage of the cell cycle. Dysfunction of any step in this regulatory cascade causes abnormal cell proliferation which underlies many human pathological conditions, such as cancer. A crucial step to understanding these conditions is the ability to understand the mechanisms underlying alterations in cell cycle progression. Enzo Life Sciences' Nuclear-ID™ Green probe provides a convenient approach for studying the induction and inhibition of cell cycle progression by flow cytometry. It is suitable for (1) determining the percentage of cells in a given sample that are in G_1/G_1, S and G_2/M phases, as well as to quantify cells in the sub-G_1 phase, and (2) DNA studies in live, permeabilized and fixed cells for normal cell lines and cell lines exhibiting multiple ploidy levels. The green probe also provides flexibility with respect to (1) sample size, (2) medium for staining, (3) concentration of probe for staining and (4) incubation temperature.

Additionally, Nuclear-ID™ Green probe provides a convenient approach for analysis of late stage apoptosis by flow cytometry and fluorescence microscopy. When incubated with Nuclear-ID™ Green probe, the compacted chromatin of apoptotic cells take up increased amounts of stain compared to the healthy cells. The Nuclear-ID™ Green probe can be used in microplate assays in HTS format as well.

Several small molecules permeable to cells are known that affect the progression of cell cycle in a specific manner. We have examined the effect of a range of such molecules, available from Enzo Life Sciences, on the phases of cell cycle as well as cellular health.

Thus the Nuclear-ID™ Green probe is suitable for (1) Analysis of cell cycle modulation by various drugs and (2) differentiating between healthy and apoptotic cells with condensed nuclei on various instrument platforms.

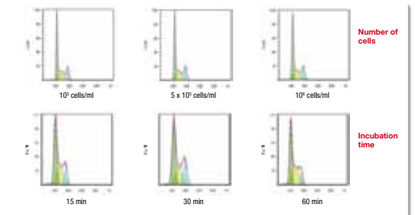

FIGURE 2: Cell cycle results independent of cell concentration and incubation time.

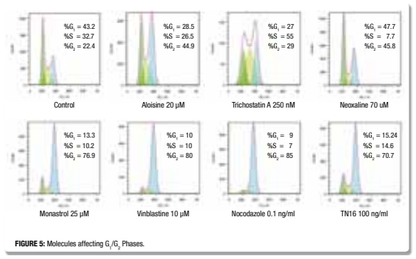

FIGURE 5: Molecules affecting G_1/G_1 Phases.

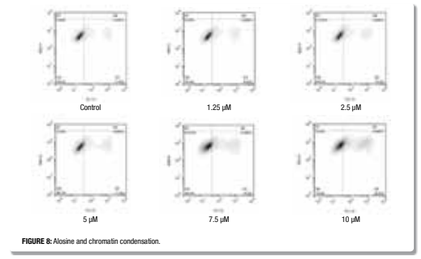

FIGURE 8: Aloisine and chromatin condensation.

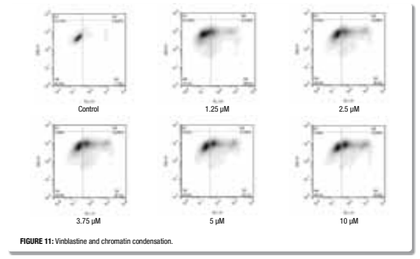

FIGURE 11: Vinblastine and chromatin condensation.

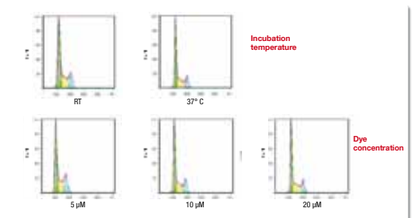

FIGURE 3: Cell cycle results independent of incubation temperature and dye concentration.

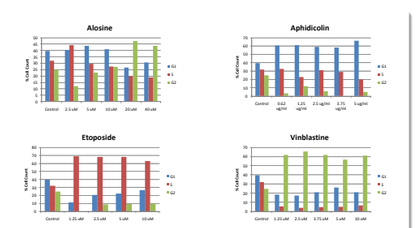

FIGURE 6: Cellular dose response to selected compounds.

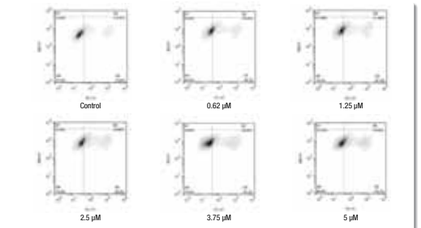

FIGURE 9: Aphidicolin and chromatin condensation.

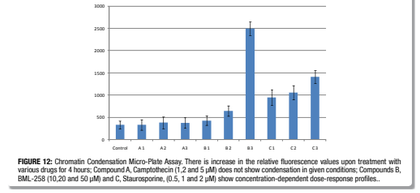

FIGURE 12: Chromatin Condensation Micro-Plate Assay. There is increase in the relative fluorescence values upon treatment with various drugs for 4 hours; Compound A, Camptothecin (1,2 and 5 µM) does not show condensation in given conditions; Compounds B, BML-258 (10,20 and 50 µM) and C, Staurosporine, (0.5, 1 and 2 µM) show concentration-dependent dose-response profiles..

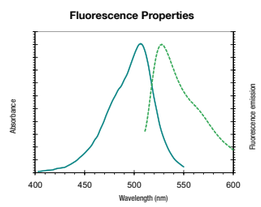

FIGURE 1: Absorption-Emission Spectra of Nuclear-ID™ Green Dye

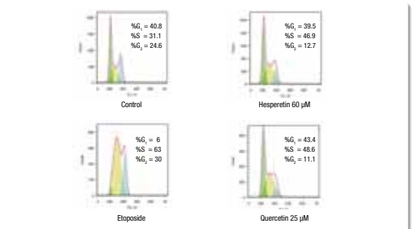

FIGURE 4: Molecules affecting S phase.

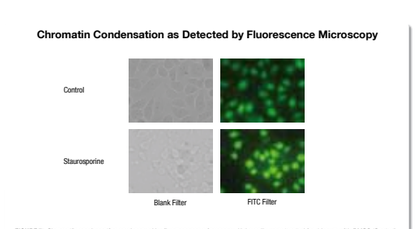

FIGURE 7: Chromatin condensation as observed by fluorescence microscopy. HeLa cells were treated for 4 hours with DMSO (Control) or 2 µM Staurosporine on a slide and stained with 5 µM Nuclear-ID™ Green dye.

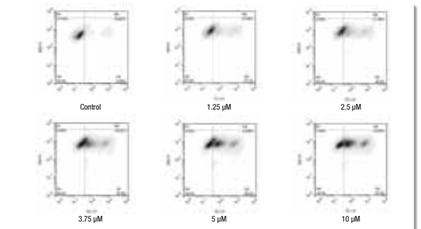

FIGURE 10: Etoposide and chromatin condensation.

CONCLUSIONS

- Nuclear-ID™ Green dye has an absorption maximum of 504 nm and emission maximum of 531 nm, making it compatible with any instrument that can detect FITC.
- The dye readily stains live, permeabilized or fixed cells.
- Nuclear-ID™ Green dye can efficiently be used for cell cycle analysis.
- Nuclear-ID™ Green dye can be used to study molecules affecting cell cycle progression
- Nuclear-ID™ Green dye detects changes in chromatin structure arising from apoptosis as a ~50-fold increased fluorescence in the apoptotic nuclei.

Reagents and Kits used in This Study

Product	Prod. No.
Nuclear-ID™ Green Cell Cycle Kit for flow cytometry	ENZ-51004-100
Nuclear-ID™ Green Chromatin Condensation Detection Kit for fluorescence microscopy and flow cytometry	ENZ-51021-K200
Aloisine A	ALX-270-395
Aphidicolin	BML-CC101
Hesperetin	ALX-385-011
Monastrol	GR-122
Nocodazole	ALX-350-409
Quercetin dihydrate	ALX-385-001
Vinblastine	ALX-350-257
TN-16	T-120
Trichostatin A	GR-309
Nocodazole	BML-T101
Etoposide	BML-GR307

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