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PULSAR-Z: A New Cell-Permeable Dye For Cell Cycle Analysis Using Flow Cytometry And Laser Scanning Cytometry at 488 nM.

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Abstract

Pulsar-Z is a new fluorogenic dye that has been developed by Biosearch Technologies, Inc. for staining nucleic acids in multiple formats. The dye has an excitation wavelength of 400-500 nm and emission of 575-625 nm. The dye can be used to perform cell cycle analysis by flow cytometry and laser scanning cytometry with or without other fluorescent dyes. It can be used to stain chromosomes in standard metaphase spreads, stain nucleic acids resolved by gel electrophoresis, and to visualize nuclei by standard fluorescence microscopy.

Introduction

The use of nucleic acid dyes in todays laboratories is widespread and encompasses many techniques including fluorescent microscopy, flow cytometry, laser scanning microscopy, genetic analyzers, and staining of gel matrices, to name a few.

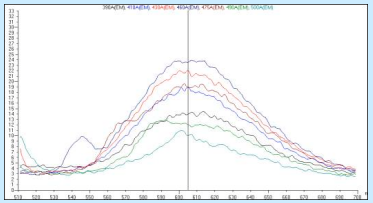
Of particular interest is cell cycle analysis using flow cytometry or laser scanning cytometry which can be used as a tool for studying cell signaling, control of cell growth and regulation, and apoptosis. The "cell cycle" is a common designation for those processes that occur in sequence during cell growth and division. In normal proliferating cells, four distinct phases of the cell cycle have been recognized: G_1 (gap phase 1), S (DNA synthesis phase), G_2 (gap phase 2), and M-phase (mitosis).

Cell cycle analysis is a routine procedure in many laboratories. However, many cytometers utilize a single wavelength argon laser at 488 nm and can not take advantage of the few cell-permeable DNA dyes available. Due to this shortcoming, researchers must rely on methods to permeabilize cell membranes prior to DNA staining which causes deliberate cell death, alters cellular components, and is time consuming. In this study, we have synthesized a dye (Pulsar-Z) that excites at 488nm and is *self-permeabilizing to many cell types* and can be used in a variety of nucleic acid staining techniques.

Materials and Methods

Pulsar-Z was synthesized at Biosearch Technologies, Inc. Nucleic acid staining was performed using a final Pulsar Z concentration of 0.03 mg/ml ($450^{\lambda b}$nm=0.033) in a buffer containing 65uM sodium citrate, 0.8 U/ml RNase A, and suspended in a base solution of 1 x PBS. Samples were analyzed using a Beckman Coulter flow cytometer and a CompuCyte laser scanning cytometer. Fluorescent microscopy was performed using a Zeiss Axioskop with a 485 excitation and 510 LP emitter. Agarose and polyacrylamide gels were performed using standard analytical techniques. Chromosomal spreads were performed using a colcemid block and dropping mitotic cells on to a slide to generate a standard metaphase spread.

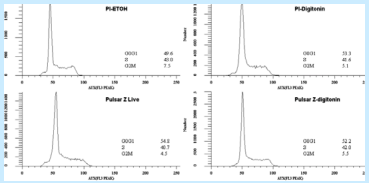
Emission Spectra of Pulsar-Z as Tested



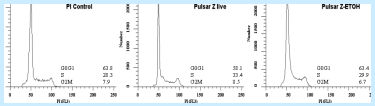
Flow Cytometry Data Comparing Pulsar-Z to Propidium Iodide

Cells were cultured, harvested, stained, and analyzed simultanously. Cell cycle ratios are express in percent of total.

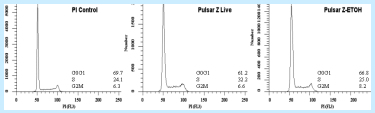
Suspended Cells -36x4 human lymphoblast B cell line



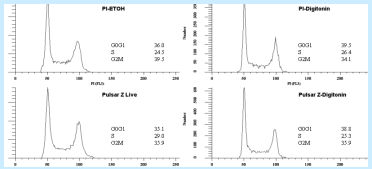
Suspended Cells -L1210 Mouse B cell



Adherent Cells -Prostate DU145



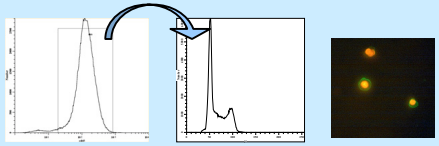
Adherent Cells -Baby Hamster Kidney



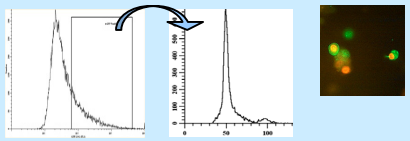
Results

Pulsar-Z Is Compatible With Other Fluorescent Markers

L1210 cells were stained with a rabbit anti-mouse CD45 and a secondary of goat anti-rabbit AlexaFluor 488. Pulsar-Z was then used as an indicator of cell cycle of CD45+ cells.



Human C10 lung cells transfected with GFP and stained used Pulsar-Z for cell cycle.



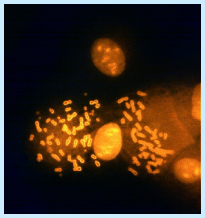
Summary of Staining Results for Cell Lines evaluated with Pulsar-Z

Cell Type	Adherent or Suspended	Ethanol Permeabilized	Digitonin Permeabilized	Live Cells
HEK 293 [Kidney]	A	Good	ND	Good
Mouse L1210 cells	S	Good	Good	Fair/Poor[1]
U2OS [Osteocytes]	A	Good[1]	Good	Good
Chinese hamster ovarian	A	Good	ND	Good
NIH 3T3 fibroblasts	A	Good[1]	Good	Good
36x4 [B-cells]	S	Good	Good	Fair[1]
TSU cells [bladder cancer]	A	ND	ND	Fair
T-cells [human]	S	Good[1]	Poor	Good
Epithelial Lung C10	A	Fair[1]	Good	Good
Primary T lymph [human]	S	ND	ND	Good
Luc8-Epithelial lung cancer	A	Good	ND	Good
BHK cells	A	Fair	Good	Good
DU145 Prostate	A	Good	ND	Good

[1] Note: 1.5 x concentration required for successful staining

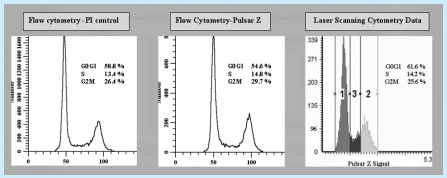
Pulsar-Z Staining of Chromosomes in a Standard Metaphase Spread

Metaphase spreads were prepared from Chinese hamster ovary cells by performing a mitotic block using colcemid. Cells were applied to a clean microscope slide, stained with Pulsar-Z for 15 minutes, and imaged 1000 x magnification (excitation 485, emitter 510 LP).

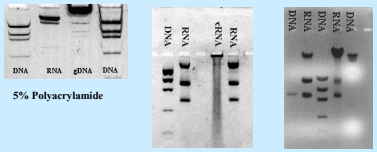


Laser Scanning Cytometry Data

BHK cells stained with Pulsar-Z, analyzed on a CompuCyte laser scanning cytometry, and compared to flow cytometric data.



Pulsar-Z Used in Post Staining of Agarose and Polyacrylamide Gels



5% Polyacrylamide

Pulsar-Z 1% Agarose

Ethidium Bromide 1% Agarose

Conclusions

- **Pulsar Z is a new patent pending nucleic acid dye that has demonstrated successful nucleic acid staining of both unpermeabilized and permeabilized cell lines for cell cycle analysis using flow cytometry and laser scanning cytometry.**
- **The dye demonstrates comparable cell cycle staining results (G_1,S,G_2 ratios) when compared to propidium iodide.**
- **The dye can intercalate both RNA and DNA.**
- **Multi-color staining strategies with Pulsar-Z including fluorescent proteins are successful.**
- **Post staining of polyacrylamide and agarose gels is easily accomplished with little background fluorescence common to ethidium bromide.**
- **The dye can be used to stain paraformaldehyde-fixed cells in many cell types (data not shown).**
- **Detergent permeabilization (digitonin) is favored over alcohol permeabilization for some cell types when required.**
- **Nucleic acid bands recovered from agarose gel matrices appear to allow for downstream analyses such as DNA sequencing. Further work is required to support our preliminary findings.**
- **Adherent cells demonstrate superior cell cycle staining over suspended cell lines. However, future modification to the dye chemistry should overcome this shortcoming.**
- **The dye appears to be self-permeabilizing and viability of stained cells is unknown at this time, however, exposure to the dye for several hours followed by reculturing yields successful growth.**

Acknowledgements

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