A cell-permeant dye for cell cycle analysis by flow and laser-scanning microplate cytometry

Enzo Life Sciences Nuclear-ID™ Red DNA stain is useful for staging cells on the basis of their DNA content. The stain can be applied to live, detergent-permeabilized or fixed cells for quantification of their distribution among the three main phases of the cell cycle (G_0/G_1, S and G_2/M). The far-red emission of the dye facilitates multiplexing with GFP and other fluorescent probes. With the dye, cells may be analyzed by fluorescence microscopy, flow cytometry and laser-scanning cytometry.

The ability to identify cell cycle position through DNA content analysis is fundamental to investigations of cell cycle–regulated protein expression and assessment of perturbations in cell cycle progression. Hoechst 33342 is one of the few live-cell DNA stains used in cell cycle analysis. However, this dye requires UV-light excitation, and many laboratory flow cytometers are equipped only with a 488 nm and/or 633 nm laser. Few cell-permeable DNA dyes are excitable at these wavelengths, forcing reliance on time-consuming methods that require cell fixation and permeabilization. Propidium iodide staining of DNA in fixed cells has been widely adopted in cell cycle analysis, but it stains all double-stranded nucleic acids, so cells must be treated with RNase before staining[1,2].

Nuclear-ID Red DNA stain is a novel DNA-intercalating dye that freely enters live cells, allowing cell cycle analysis without laborious permeabilization and fixation steps. Since the dye intercalates exclusively into double-stranded DNA, no RNase treatment is required and the fluorescence intensity of stained cells is directly proportional to their DNA content.

Cell cycle analysis by flow cytometry

With respect to live-cell analysis of cell cycle, Nuclear-ID Red DNA stain has similar performance characteristics to Hoechst 33342 stain. In untreated cells, the G_0/G_1 peak coefficient of variation is generally less than 10%, and the G_2/G_1 mean fluorescence ratio is roughly 1.8. Unlike Hoechst, the Nuclear-ID Red DNA stain is excited by either 488-nm or 633-nm laser sources. Staining is easy to perform: simply add the dye, incubate and analyze. The assay has been validated using a wide range of cell types, cell concentrations, dye concentrations, staining times and temperatures. Nuclear-ID Red DNA stain is especially suitable for monitoring drug-induced changes in cell cycle dynamics (**Fig. 1**). We exposed Jurkat cells to 0.1 μg ml^{-1} nocodazole, 3 μM etoposide or 30 μg ml^{-1} aphidicolin. Then we incubated the cells with a 1:250 dilution of Nuclear-ID Red DNA stain in medium and assayed the cultures using the FL3 channel of a FACSCalibur flow cytometer (BD Biosciences), equipped with a 488-nm laser. Flow cytometric analysis revealed the dye's ability to accurately profile DNA content and cell cycle distribution in cells subjected to these treatments.

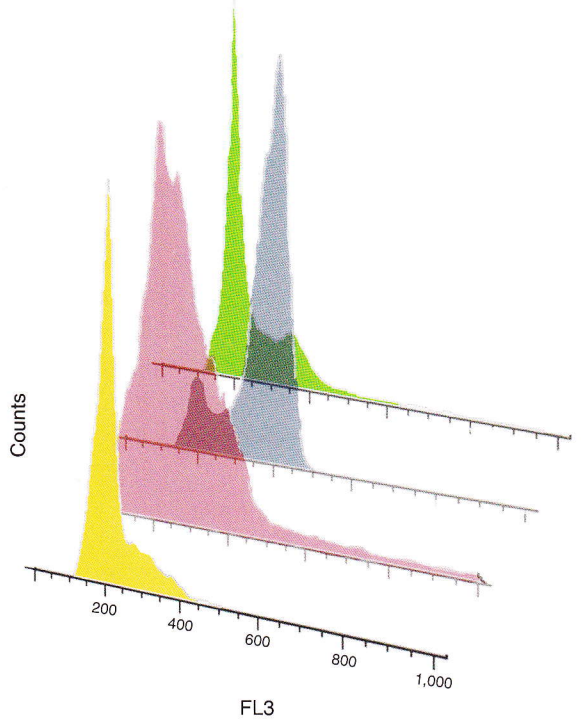

Figure 1 | Determining drug effects on the cell cycle by flow cytometry. Analysis of control Jurkat cells (green; top axis), nocodazole-treated cells, which accumulate in G_2/M phase (blue), etoposide-treated cells, which accumulate in S phase (red) and aphidicolin-treated cells, which accumulate in G_0/G_1 phase (yellow; bottom axis). A FACSCalibur flow cytometer was used in these experiments.

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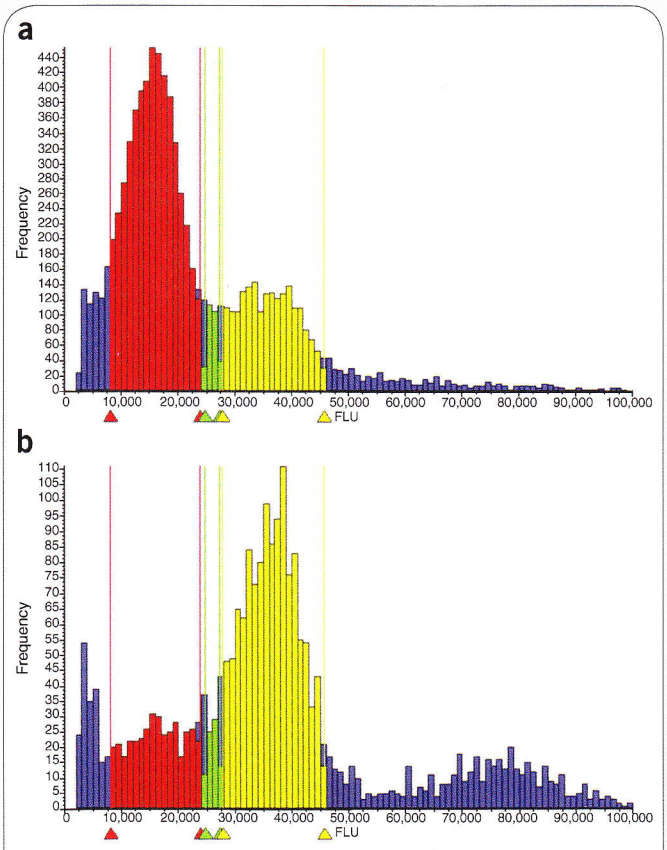

Figure 2 | Nocodazole-mediated cell cycle arrest of adherent HeLa cells, as measured using a laser-scanning microplate cytometer (Acumen ^{e}X3). (**a**,**b**) Cell population in various stages of the cell cycle are indicated: sub-G0 phase (blue, left); G0/G1 phase (red); S phase (green); and G2/M phase (yellow). Blue regions on the right correspond to cell doublets. Analysis of control cells (**a**) and nocodazole-treated cells (**b**).

Cell cycle analysis by laser-scanning microplate cytometry

The inability of flow cytometry to analyze adherent cells *in situ*, its requirement for relatively high densities of cells (10^5–10^6 cells per milliliter) and its inherently low-throughput nature has provided an impetus for development of alternative instrument platforms, such as laser-scanning microplate-based cytometry[3]. Using Nuclear-ID Red DNA stain with a microplate cytometer allows all cell processing steps to be performed in microplates. Scientists at TTP Labtech grew cells in culture medium alone or in culture medium with 0.2 μg ml^{-1} nocodazole (**Fig. 2**). A 1:1,000 dilution of Nuclear-ID Red DNA stain in 0.2% Triton X-100–containing phosphate-buffered saline was added after ~20-hour drug incubation, and plates were scanned on an Acumen ^{e}X3 fluorescence microplate cytometer using the instrument's 633-nm laser. Cell cycle analysis of adherent HeLa cells using this instrument showed excellent correlation with results achieved by flow cytometry.

Multiplexing with GFP

Nuclear-ID Red DNA stain is a far-red–emitting dye (emission maximum, 640 nm) that provides minimal spectral overlap with GFP or

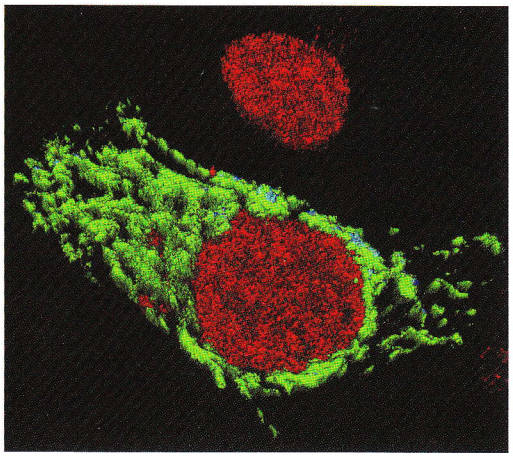

Figure 3 | The spatial relationship between GFP-expressing mitochondria and nuclei labeled with Nuclear-ID Red DNA stain in living cells, highlighted using a structured illumination method (Apotome). Optical sections were used to create a three-dimensional reconstruction of the cell.

with antibody conjugates of fluorescein (FITC), DyLight 488 (Thermo-Fisher Scientific) and Alexa Fluor 488 (Invitrogen Corporation). As the dye is highly resistant to photobleaching, it is suitable for wide-field and confocal fluorescence microscopy. We detected the nucleus with Nuclear-ID Red dye in GFP-expressing HeLa cells, containing a TurboGreen-tagged mitochondrial targeting sequence of human cytochrome c oxidase subunit VIII precursor (Marinpharm GmbH) (**Fig. 3**). Cells were stained with a 1:1,000 dilution of Nuclear-ID Red DNA stain. The Nuclear-ID Red dye was imaged using a Texas Red filter set, and the green fluorescence was imaged using a FITC filter set, on an Axio Observer Z1 wide-field fluorescence microscope equipped with an Apotome slider module (Zeiss).

Conclusions

The principal advantage of Nuclear-ID Red DNA stain in live cells is that the assay protocol is simple, as there is no need for fixation or RNase treatment. Nuclear-ID Red DNA stain is compatible with a broad range of instruments, including conventional and confocal fluorescence microscopes, as well as flow cytometers and microplate-based cytometers. Its far-red emission makes the stain ideal for multiplexing with common fluorescent probes, such as GFP.

ACKNOWLEDGMENTS

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