

The APO-BrdU™ TUNEL Assay Kit includes complete protocols for use in flow cytometry applications, though it may also be adapted for use with fluorescence microscopy. Each kit contains:

- Terminal deoxynucleotidyl transferase (TdT), for catalyzing the addition of BrdUTP at the break sites
- 5-Bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP)
- Alexa Fluor® 488 dye-labeled anti-BrdU mouse monoclonal antibody PRB-1, for detecting BrdU labels
- Propidium iodide/RNase staining buffer, for quantitating total cellular DNA
- Reaction, wash and rinse buffers
- Positive control cells (a fixed human lymphoma cell line)
- Negative control cells (a fixed human lymphoma cell line)
- Detailed protocols

Sufficient reagents are provided for approximately 60 assays of 1 mL samples, each containing approximately  $1 \times 10^6$  cells/mL.

### Detecting DNA Strand Breaks with ChromaTide® Nucleotides

Break sites have traditionally been labeled with biotinylated dUTP, followed by subsequent detection with an avidin or streptavidin conjugate<sup>37–40</sup> (Section 7.6, Table 7.9). However, a more direct approach for detecting DNA strand breaks in apoptotic cells is possible via the use of our ChromaTide® BODIPY® FL-14-dUTP (C7614) as a TdT substrate<sup>41,42</sup> (Figure 15.5.9). The single-step BODIPY® FL dye-based assay has several advantages over indirect detection of biotinylated or haptenylated nucleotides, including fewer protocol steps and increased cell yields. BODIPY® FL dye-labeled nucleotides have also proven superior to fluorescein-labeled nucleotides for detection of DNA strand breaks in apoptotic cells because they provide stronger signals, a narrower emission spectrum and less photobleaching<sup>41</sup> (Figure 15.5.9).

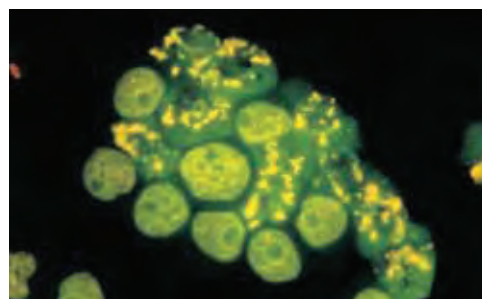
*In situ* DNA modifications by labeled nucleotides have been used to detect DNA fragmentation in what may be apoptotic cells in autopsy brains of Huntingtons and Alzheimer disease patients.<sup>43–46</sup> DNA fragmentation is also associated with amyotrophic lateral sclerosis.<sup>47</sup>

Analogous to TdT's ability to label double-strand breaks, the *E. coli* repair enzyme DNA polymerase I can be used to detect single-strand nicks,<sup>48,49</sup> which appear as a relatively early step in some apoptotic processes.<sup>50–52</sup> Because our ChromaTide® BODIPY® FL-14-dUTP (C7614) and ChromaTide® fluorescein-12-dUTP<sup>53,54</sup> (C7604) are incorporated into DNA by *E. coli* DNA polymerase I, they are also effective for *in situ* labeling with the nick translation method.<sup>55</sup>

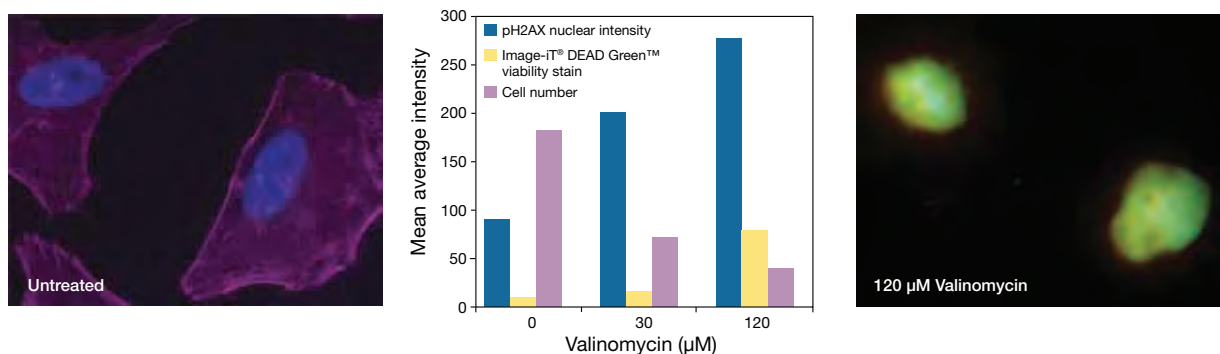
### High-Content Analysis of Genotoxicity and Cytotoxicity

In mammalian cells, a double-strand break (DSB) in genomic DNA is a potentially lethal lesion. One of the earliest known responses to DSB formation is phosphorylation of H2A histones. Specifically, DNA damaging agents induce phosphorylation of histone variant H2AX at Ser139, leading to the formation of DNA foci at the site of DNA DSBs.<sup>56–58</sup>

The HCS DNA Damage Kit (H10292) was developed to enable simultaneous quantitation of two cell health parameters, genotoxicity and cytotoxicity, by high-content analysis in the same cell (Figure 15.5.10). DNA damage is measured as an indication of genotoxicity and accomplished by specific antibody-based detection of phosphorylated H2AX (Ser139) in the nucleus. Cytotoxicity is measured with the Image-iT® DEAD Green™ viability stain (also available as a stand-alone reagent, I10291),



**Figure 15.5.9** HL-60 cells treated with camptothecin for 3 hours. The DNA strand nicks characteristic of apoptosis were detected with the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) assay using the fluorescently labeled nucleotide ChromaTide® BODIPY® FL-14-dUTP (C7614). Image contributed by Zbigniew Darzynkiewicz, Cancer Research Institute, New York Medical College.



**Figure 15.5.10** Detection of genotoxicity and cytotoxicity in valinomycin-treated A549 cells using the HCS DNA Damage Kit (H10292). A549 cells were treated with 30 μM or 120 μM valinomycin for 24 hr before performing the assay. With increasing concentrations of valinomycin, cells showed genotoxic effects as indicated by detection with a pH2AX antibody in conjunction with Alexa Fluor® 555 goat anti-mouse IgG antibody (orange fluorescence), and cytotoxic effects as indicated by staining with the Image-iT® DEAD Green™ viability stain (green fluorescence). Blue-fluorescent Hoechst 33342 was used as a nuclear segmentation tool, and Alexa Fluor® 647 phalloidin was used to visualize F-actin (pseudocolored magenta). The image on the left shows untreated cells with intact F-actin cytoskeletons and no evidence of cytotoxicity or genotoxicity. The image on the right shows cells treated with 120 μM valinomycin, which completely disrupted the actin cytoskeletons, increased levels of DNA damage and compromised plasma membrane integrity.