

2.2.3 Assays that use DNA stains

One can differentiate between three methods for studying cell death that use DNA stains: dye exclusion method, profile of DNA content, morphological changes.

Dye exclusion method

Viable (intact plasma membrane) and dead (damaged plasma membrane) cells can be discriminated by differential staining. Cells with disturbed plasma membrane permeability are stained, whereas undamaged (viable) cells are not stained with dyes that do not penetrate the plasma membrane (“exclusion dyes”). The most frequently used dye for exclusion tests is trypan blue. In addition, the fluorescent dye, propidium iodide (PI) which becomes highly fluorescent after binding to DNA, can be used in the same manner. The stained and unstained cells are counted with a standard light microscope (trypan blue), or flow cytometer (PI) (Table 9).

Profile of DNA content

If cells are permeabilized, the LMW DNA inside the cytoplasm of apoptotic cells leaks out during the subsequent rinse and staining procedure. The lower DNA content of these cells means they contain less DNA stained by the fluorochrome. Thus, cells with lower DNA staining than that of G_1 cells (the so-called “sub- G_1 peaks”, “ A_0 ” cells) have been considered apoptotic. The reduction in staining/DNA content of these cells is measured by flow cytometry (Figure 37). The major disadvantage of this technique is that apoptotic G_2 -Phase cells exhibit a reduced DNA content, which could represent the DNA content of a G_1 -cell. Therefore it may not be detected as apoptotic. This would result in an underestimation of the apoptotic population.

DNA-binding dyes (Fluorochromes)	Dye enters		Dye stains	
	Viable cells	Non viable cells	Nucleus (DNA)	Cytoplasm (RNA)
Acridine orange	Yes	Yes	Green	Red-orange
Hoechst 33342	Yes	Yes	Blue	No
Hoechst 33258	No	Yes	Blue	No
DAPI	No	Yes	Bright blue	No
Ethidium bromide	No	Yes	Orange	Slightly red
Propidium iodide	No	Yes	Red	No

▲ Table 9: Common fluorochromes used to stain the genomic DNA of viable and/or non-viable cells.

