

2.2.4 Summary of methods for studying apoptosis in individual cells

| Method/Roche Applied Science product | Parameter analyzed | Assay principle | Advantages | Limitations | For product information, see |
|---|--|--|---|--|------------------------------|
| Staining of chromosomal DNA after permeabilization (DNA content) ^{22, 32} DAPI, propidium iodide* | DNA fragmentation (HMW DNA, DNA content) | <ul style="list-style-type: none"> Apoptotic cells are permeabilized with ethanol or detergent. During this procedure the LMW DNA inside the apoptotic cell leaks out and is removed during the subsequent washing steps. The HMW DNA retained in the cells is stained with a DNA binding dye such as propidium iodide. The amount of HMW DNA is quantified by flow cytometry ("sub G₁" or "A₀" peak). | <ul style="list-style-type: none"> Quick and cheap Minimal overlap between the peak representing apoptotic ("sub G₁") and normal G₁ cells Estimation of position in cell cycle allows cell cycle specificity of apoptosis to be studied Applicability to any DNA fluorochrome and instrument | <ul style="list-style-type: none"> Degree of extraction of LMW DNA during the washing and staining procedure not always reproducible Not specific for apoptosis: Sub G₁ peak can also represent mechanically damaged cells, cells with different chromatin structure, or normal cells with lower DNA content in heterogeneous cell populations May not detect cells induced to apoptosis in G₂ No discrimination of apoptotic cells from dead cells which have lost their membrane integrity | page 49 of this guide |
| Staining of chromosomal DNA | Chromatin morphology | <ul style="list-style-type: none"> Apoptotic cells are stained by the addition of DNA fluorochromes which are able to cross the intact plasma membrane, such as acridine orange. Can be done with DAPI on fixed cells. The stained DNA allows the altered morphology of the nuclear chromatin to be visualized by fluorescence microscopy. | <ul style="list-style-type: none"> Quick and cheap Discrimination between viable and dead cells when counterstained with propidium iodide using vital dyes (acridine orange, Hoechst 33342) Simultaneous staining of cell surface antigens with standard fluorescein and phycoerythrin conjugates possible if Hoechst 33342 is combined with 7-amino-actinomycin D | <ul style="list-style-type: none"> No quantitative measurement Subjective: no clear cut-off point between normal and apoptotic cells Clear morphologically distinct apoptotic nuclei appear late during apoptosis: May lead to an underestimation of apoptotic cells | |
| Active labeling of cells by nick translation (ISNT) ³⁴ | DNA strand breaks (nicks) and DNA fragmentation (staggered DNA ends) | <ul style="list-style-type: none"> Apoptotic cells are fixed with formaldehyde and subsequently permeabilized. DNA strand breaks are labeled with modified nucleotides using exogenous DNA polymerase (nick translation). The incorporated nucleotides are visualized with a secondary detection system which has a reporter molecule (e.g. fluorescein, AP, POD). | <ul style="list-style-type: none"> Counterstaining with DNA fluorochrome (profile of DNA content) allows cell cycle specificity of apoptosis to be studied (only by flow cytometry) Identification of apoptosis at a molecular level (DNA strand breaks) Suitable for tissue sections | <ul style="list-style-type: none"> Labor-intensive and time-consuming; only a few tests may be performed simultaneously Undefined cell loss during fixation procedure (loss of specific cell population?) Many cells (2–5 x 10⁶/test) required | |
| Active labeling of cells by end labeling (TUNEL) ^{20, 35} <i>In Situ</i> Cell Death Kit, Fluorescein, TMR, AP, POD | DNA strand breaks (nicks) and DNA fragmentation (staggered DNA ends) | <ul style="list-style-type: none"> Apoptotic cells are fixed with formaldehyde and subsequently permeabilized. DNA strand breaks are labeled with modified nucleotides using exogenous terminal transferase (end labeling). The incorporated nucleotides are visualized directly (e.g. Fluorescein-dUTP) or with a secondary detection system which has a reporter molecule (e.g. fluorescein, AP, POD). | <ul style="list-style-type: none"> Advantages like <i>in situ</i> nick translation; in addition: <ul style="list-style-type: none"> More sensitive: maximum intensity of labeling (cell staining) of apoptotic cells is higher compared to ISNT Fast kinetics of dUTP incorporation in comparison with the DNA polymerase method: 30 min is sufficient for the labeling reaction Higher sensitivity for apoptosis: TUNEL preferentially labels apoptosis in comparison to necrosis Direct detection possible by the use of Fluorescein-dUTP (without secondary detection system) for maximum sensitivity and minimal background With the direct TUNEL labeling assay less working steps are required than with the indirect assay. | <ul style="list-style-type: none"> Labor-intensive and time-consuming; only a few tests may be performed simultaneously Many cells (2–5 x 10⁶/test) required Undefined cell loss during fixation procedure (loss of specific cell population?) | pages 36–42 of this guide |
| Detection of translocated membrane component ²⁷ Annexin-V-FLUOS Annexin-V-Biotin Annexin-V-Staining Kit Annexin-V-Alexa 568 | Detection of phosphatidylserine on surface of apoptotic cells | <ul style="list-style-type: none"> Apoptotic cells are incubated with an assay protein (e.g. annexin-V) conjugated to a reporter molecule. Assay protein binds a membrane component (e.g. phosphatidylserine) found on the outer surface of apoptotic cells only. A DNA stain is added to distinguish necrotic cells (permeable) from apoptotic cells (impermeable). Apoptotic cells are made visible by assay of reporter molecule in flow cytometer or under a microscope. | <ul style="list-style-type: none"> Unique marker for apoptosis related plasma membrane changes Allows analysis by flow cytometry fluorescence microscopy, or light microscopy Allows simultaneous labeling of other cell surface antigens Annexin-V-Biotin allows fixation following Annexin-V binding for further analysis of additional cellular parameters | <ul style="list-style-type: none"> Not specific for apoptosis: Annexin-V can stain inner membrane of ruptured cells; must distinguish apoptotic from necrotic cells with an additional DNA stain Many cells (10⁶/test) required Cannot be used on tissue sections or any fixed samples | pages 44–48 of this guide |
| Trypan Blue Exclusion Assay | Damage/leakage of plasma membrane | <ul style="list-style-type: none"> Cells are incubated with dye. Dead cells take up dye; living cells do not. Stained (dead or damaged) cells are determined under a light microscope. Dye binds to intracellular proteins of leaky cells. | <ul style="list-style-type: none"> The classical standard method to distinguish viable from dead cells by light microscopy Quick and cheap Only a small fraction of total cells from a cell population is required | <ul style="list-style-type: none"> Each individual sample has to be counted: only a few tests may be performed simultaneously Subjective evaluation Not suitable for detection of apoptosis Stains only necrotic cells or very late apoptotic cells (secondary necrosis) | |
| Propidium Iodide Exclusion Assay Propidium iodide solution* | Damage/leakage of plasma membrane | <ul style="list-style-type: none"> Cells are incubated with fluorescent dye. Dead cells take up dye; living cells do not. Stained (dead or damaged) cells are determined under a microscope or by flow cytometry. Dye binds to DNA of leaky cells. | <ul style="list-style-type: none"> The standard method to distinguish viable from dead cells by fluorescence microscopy and flow cytometry Double labeling procedures possible: simultaneous detection of e.g. surface antigens Quick and cheap Only a small fraction of total cells from a cell population is required | <ul style="list-style-type: none"> Each individual sample has to be counted: only a few tests may be performed simultaneously Not specific for apoptosis | page 49 of this guide |

*Sold only in the US

▲ Table 11: Methods for studying apoptosis in individual cells.