

DAPI Reagent

DAPI Staining Kit

CATALOG # AAA106122	APPLICATIONS Immunofluorescence
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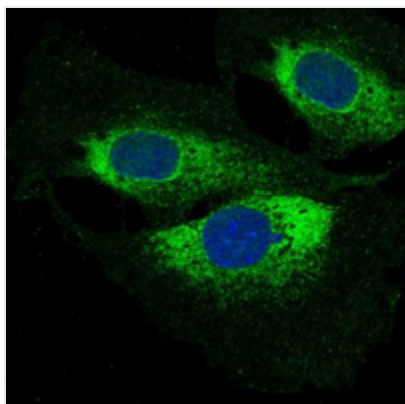
PRODUCT SYNONYMS

DAPI; DAPI Staining Kit; DAPI reagent

PRODUCT SPECIFICATIONS

Applicable Applications for DAPI reagent	IF (Immunofluorescence)
Preparation and Storage	Store at 4 degree C.

IMAGES



IF (Immunofluorescence)

Immunofluorescent analysis staining in HeLa cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a humidified chamber. Cells were washed with PBST and incubated with a FITC-conjugated secondary antibody (green) in PBS at room temperature in the dark. DAPI was used to stain the cell nuclei (blue).

Related Product Information for DAPI reagent

Background/Introduction: Cytolytic activity is an important process for eliminating intracellular pathogens and cancer cells. This process is accomplished through various immune effector mechanisms including natural killer (NK) leukocytes. NK activity is facilitated by non-specifically lysing infected targets through the use of NK receptors, or the FcγRIII (CD16) receptor, recognizing IgG bound to specific antigens on the target cell surface¹. NK cells may also induce apoptosis in target cells. The activity of natural killer cells, and their effect on target cells, is frequently studied in immunomodulation experiments. Older methods to assess NK cytolytic activity include measuring the release of lactate dehydrogenase, and more commonly, the release of radioactive ⁵¹Cr from lysed target cells¹. Unfortunately, these techniques have several drawbacks. Traditional enzyme-release assays are often skewed by the large number of necrotic effector cells. Problems associated with ⁵¹Cr release methods include high spontaneous leakage resulting in high backgrounds, high cost, short half-life, and the health risks due to exposure to radioactive material². Beyond these limitations, these assays frequently underestimate the true level of cytotoxicity, as they are unable to detect early-stage apoptotic cells. Flow cytometric assays have been developed to overcome some of the difficulties associated with older assays like lactate dehydrogenase and ⁵¹Cr release assays. Once such early version involved the detection of NK cytotoxicity activity by staining target cells with the green fluorescent dye, F-18, in combination with the DNA intercalating dye, propidium iodide³. Since then, a red fluorescent membrane dye, PKH-26, has been used in preference to F-18, and in combination with the viability probe, TO-PRO-3 iodide⁴⁻⁷. However, despite correlations of greater than 95% when compared with the ⁵¹Cr release assay¹, the PKH-26 method is problematic. It is difficult to use at a constant concentration, leading to unreliable staining, and the staining procedure requires multiple steps, often decreasing the viability of the target cells. More recently, the problems with older flow cytometric assays were overcome with the use of 5(6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE), a green fluorogenic reagent that diffuses into target cells and covalently binds to primary amino groups on intracellular molecules⁸. Intracellular esterases quickly cleave the acetate groups from the dye, thus converting it to its green fluorescent form. Any unbound reagent diffuses back out of the cell. Building upon these techniques, [stage.aaabiotech.com](https://stage.aaabiotech.com/reagent/dapi/106122) has developed the Total Cytotoxicity & Apoptosis Assay, a flow cytometric assay combining the green fluorescing cellular stain, (CFSE), with a red fluorescing live/dead stain, 7-aminoactinomycin D (7-AAD), and SR-FLICA apoptosis detection reagent to concurrently quantify caspase-positive cells. The assay can be used to determine total cytotoxicity in the form of apoptosis and necrosis. It will quantify 4 populations of cells: live; early apoptotic; late apoptotic; and necrotic cells within a single sample tube. While other methods often underestimate the true level of cytotoxicity, Total Cytotoxicity & Apoptosis Assay is the best method to accurately quantify cell death because it can detect cells in early apoptosis. This often reveals a significant percentage of cells that are 7-AAD negative (indicating that they are alive and do not have compromised membranes), but are SRFLICA positive (meaning that they are becoming apoptotic and dying and have active caspase enzymes). In the assay, CFSE is first used to label the target cell population green (Figure 4). The unstained effector cells are then added and incubated with the target cells (referred to as the Effector:Target, or 'E:T' mixture, Figure 5). As all the target cells are initially labeled with green fluorescing CFSE, and the effector cells are not, these two populations can be easily distinguished (Figure 8). Apoptotic target cells can then be identified by labeling with the second reagent, SR-FLICA (Figure 6). SR-FLICA is an orange/red fluorescent poly caspase inhibitor, SR-VAD-FMK, which binds to active caspase enzymes up-regulated for apoptosis⁹. Upon completion of the E:T incubation (which includes exposure to the apoptosis detection reagent), the last reagent, 7-AAD, is added to stain all dead cells red by binding to the DNA of membrane-compromised cells (Figure 7). With proper compensation and gating of the flow cytometer using the designated instrument controls (Figures 3 and 8-13) researchers can distinguish between target and effector cells, and living, necrotic, and apoptotic cells, and assess the level of cytotoxicity in their samples (Figures 14-15).

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/reagent/dapi/106122>