Ready-to-use DRAQ5 Live Cell DNA Dye (5 mM)

Cat. No.: D1427942 | Pack size: 20 µL, 50 µL, 200 µL | Storage: Store at 2-8°C, Protected from light

Overview

DRAQ5 is a far-red lipophilic and membrane-permeable DNA dye that specifically binds to DNA for nuclear staining, and is suitable for the detection of both live cells and fixed dead cells. Compared with traditional nuclear dyes (e.g., Hoechst and DAPI, the most commonly used blue fluorescent probes) which are excited in the ultraviolet (UV) region, these two dyes restrict users from co-using them with other UV-excited or blue-emitting fluorescent dyes in the same experiment. DRAQ5 emits fluorescence in the far-red region, which perfectly avoids the UV excitation and blue fluorescence channels, thus solving the limitation of dye spectral overlap in multi-channel detection. Meanwhile, DRAQ5 specifically binds to DNA but not RNA, and can be used for the analysis of DNA content during the cell cycle. In comparison with Propidium Iodide (PI) for cell cycle analysis, DRAQ5 eliminates the need for RNase digestion, cell membrane lysis and washing steps, greatly simplifying the experimental workflow, saving operational time for researchers and reducing sample loss.

With its unique spectral properties and convenient operational advantages, DRAQ5 has become a key tool for multicolor fluorescence imaging and cell cycle analysis. Its applications include: multi-channel cell labeling in combination with fluorescent proteins (e.g., GFP) or other visible light dyes to realize colocalization observation of cell structures and specific proteins; direct use for proliferation detection of cells fused with GFP fluorescent proteins, with accurate analysis of DNA content via nuclear counterstaining; efficient study of cell cycle progression to obtain accurate DNA content data without complex preprocessing. The advent of this dye has broken the application limitations of traditional staining technologies, providing a flexible and efficient solution for research such as dynamic observation of live cells and high-throughput cell analysis, and promoting the convenience and accuracy of cell-level research. For in vitro research use only. Not for clinical diagnosis or treatment.

Note: Performance equivalent to AAT ReadiUse™ DRAQ5 Staining Solution 5 mM in Water (17558).

Note: DRAQ5 in this document is a trademark and registered trademark of Biostatus Limited; ReadiUse™ in this document is a trademark and registered trademark of AAT Bioquest.

Application Scope

Live cell imaging, real-time nuclear labeling and dynamic observation of live cells, cell cycle analysis, DNA labeling of live cells, research on chromatin ultrastructure, etc.

Product Features

1. No membrane lysis/washing required: Direct staining of live cells without membrane lysis, and no washing after staining;

2. Ultra-rapid staining: Fast staining in as little as 5 minutes at room temperature, greatly shortening experimental time;

3. Easy to use: Supplied as a ready-to-handle 5 mM liquid formulation, ready for use after dilution as needed;

4. Ideal for multicolor combination: Broad excitation and far-red emission bands, suitable for multicolor fluorescent labeling combinations;

5. Cell imaging compatibility: As a membrane-permeable nuclear dye, DRAQ5 can be used for live cell imaging and nucleic acid content quantification.

Product Parameters

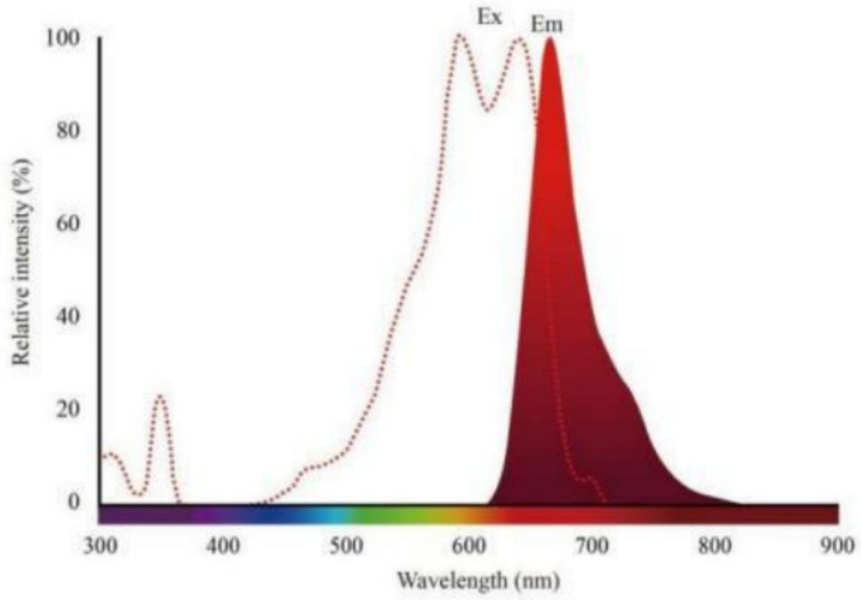
1. Ex/Em: 601/699 nm;

2. CAS No.: 252903-95-0;

3. Molecular Formula: $C_{22}H_{28}N_4O_4$;

4. Molecular Weight: 412.48;

Spectrum:



Product Components

Component	40 Tests	100 Tests	400 Tests
Ready-to-use DRAQ5 Live Cell DNA Dye (5 mM in Water)	20 µL	50 µL	200 µL

Note: Specifications are calculated based on 5×10^4 cells/well with a working staining solution concentration of 20 µM.

Precautions

1. DRAQ5 is a far-red fluorescent dye that is invisible to the naked eye and requires observation with a CCD camera or laser confocal microscope.

2. Due to the broad excitation and emission wavelength ranges of DRAQ5, it is not recommended to use DRAQ5 in combination with other far-red fluorescent dyes excitable by 488 nm or 633 nm laser.

3. Fluorescent dyes are subject to quenching; please protect from light as much as possible to slow down quenching.

4. Briefly centrifuge the product before use before performing subsequent operations.

5. This product is for research use only and shall not be stored in ordinary residential premises.

6. For your safety and health, comply with the general laboratory safety regulations of your institution.

Instructions for Use

I. Pre-Experiment Preparation

1. Reagent Preparation:

(1) Take the reagent out of storage conditions (e.g., 4 °C) and equilibrate to room temperature (15-25 °C).

(2) Prepare cell culture medium and buffer (e.g., azide-free PBS).

2. Instrument Preparation:

Fluorescence microscope: Equipped with a 633 nm or 647 nm light source.

II. Operational Procedures

Protocol 1: Fluorescence Microscopy Detection

1. Cell Preparation: Prepare azide-free PBS buffer or specific medium for specific cells. Resuspend cells with PBS or medium and control the cell density ≤4×10^5 cells/mL. Roughly estimate the cell number for adherent cells or tissues.

2. Working Staining Solution Preparation: Dilute DRAQ5 fluorescent staining solution according to Table 1. DRAQ5 staining solution can be directly added to the surface of adherent cells or tissue sections, or directly into fresh culture medium.

Notes: (1) The optimal staining concentration of DRAQ5 may vary for different cell types; it is recommended to perform a pre-experiment for cell staining initially and set up a gradient of different DRAQ5 staining concentrations to determine the optimal one.

(2) For experiments requiring continuous monitoring (e.g., EGFP-labeled protein translocation assays), DRAQ5 staining solution should be added at a concentration of 1 μM before the addition of any agonists/antagonists (usually 0.5-3 h in advance).

Table 1. Cell Number and Corresponding DRAQ5 Volume & Final Concentration

Cell Number	Volume of PBS or Medium	5 μM	10 μM	20 μM
1×10^6	2500 μL	2.5 μL	5 μL	10 μL
4×10^5	1000 μL	1 μL	2 μL	4 μL
2×10^5	500 μL	0.5 μL	1 μL	2 μL
1×10^5	250 μL	0.25 μL	0.5 μL	1 μL
5×10^4	125 μL	0.13 μL	0.25 μL	0.5 μL

3. Cell Staining

Mix gently and incubate at room temperature for 5-30 minutes. No washing step is required after incubation.

4. Detection:

(1) Flow cytometry detection: When the dye is excited at 488 nm, detection can be performed using a 685LP dichroic mirror and a 710/50 channel; when excited at 633 nm, a 660/20 channel is applicable. For cell cycle/DNA analysis applications, it is recommended to use longer-wavelength filters (e.g., a 735LP dichroic mirror and a 780/60 channel) to optimize the CV values of G1 and G2/M peaks.

(2) Fluorescence microscopy detection: Excitation with a 633 nm or 647 nm light source is recommended.

III. Result Interpretation

Qualitative Analysis (Microscopy):

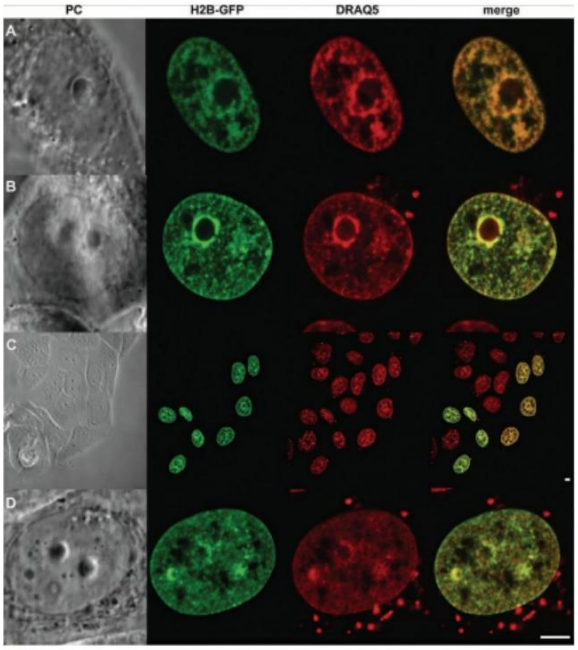


Figure 1. Fluorescence micrograph of live cell DNA staining with DRAQ5 dye

1. Normal nuclear staining: The nuclear region of live cells shows uniform red fluorescence with a clear nuclear boundary;

2. Background interference: Strong extracellular or background fluorescence indicates insufficient washing or excessively high dye concentration.

Note: The image is cited from the reference DNA labeling in living cells (doi: 10.1002/cyto.a.20172).

Frequently Asked Questions (FAQs)

1. Q: Does the DRAQ5 stock solution contain DMSO?

A: This product is an aqueous solution and contains no DMSO.

2. Q: I am performing 2-3 h live cell imaging of mouse cells with DRAQ5. How long can I image DRAQ5-stained cells before cytotoxicity occurs and causes cell death?

A: The instructions state that "DRAQ5 staining is accelerated at 37 °C, and the incubation time (5-30 min) may be shortened", but this should be determined separately by titration for each cell type. DRAQ5 can be added to the assay medium at a concentration of 1 µM for a detection duration of 0.5-3 h (typically) before the addition of any agonists/antagonists. DRAQ5 staining occurs very rapidly, so it is recommended to add DRAQ5 to live cells immediately before analysis. The incubation time with cells should not exceed 3 h, but this may vary by cell line.

3. Q: When staining the nuclei of epithelial cancer cells with DRAQ5, I found that the fluorescence becomes increasingly intense over time. This effect is more pronounced at higher laser power and with heating. What could be the cause? Does more dye bind to nuclear DNA over time? Does it only fluoresce when it reacts with nuclear DNA? Is the behavior of the dye during apoptosis known? Can the dye bind to nuclear DNA just as well when the dye concentration is low?

A: It is confirmed that DRAQ5 staining is enhanced at higher temperatures, so the incubation period may need to be adjusted. The longer the incubation period, the more signal we expect, until DNA saturation is reached. If DRAQ5 is subsequently washed away, we expect the staining to decrease slowly over time.DRAQ5 emits almost no fluorescence unless it binds to double-stranded DNA (dsDNA); it only produces a strong fluorescent signal when intercalated into dsDNA. DRAQ5 staining is not affected during apoptosis because the affinity of DRAQ5 for dsDNA is unchanged in live or dead cells. As the amount of dsDNA changes during apoptosis, the intensity of the DRAQ5 signal also changes in response to the variation of dsDNA.

4. Q: I am conducting an 18 h cell imaging experiment. When I replace the DRAQ5-containing medium with DRAQ5-free medium after 12 h, the staining intensity decreases.

① Is it necessary to keep DRAQ5 in the medium at all times, even if cytotoxic effects may occur?

② How long will the staining persist if DRAQ5 is removed from the medium?

A: ① We expect cell staining to decrease after medium replacement. Although DRAQ5 has a high affinity for dsDNA, an equilibrium exists, and the removal of low residual concentrations in the medium disrupts this equilibrium.

② We believe the staining may persist for 1-2 h after removal, but this may depend on the concentration the cells were previously exposed to. If there is any evidence of toxicity, one would want to maintain a lower concentration (possibly 0.5-1 μM). Certainly, whether DRAQ5 exerts cytotoxic effects depends entirely on the cell line and its state. Based on literature reports, it can be reasonably predicted that non-proliferating cells are less sensitive to any cell-permeable DNA intercalating probe. For example, it is more likely to be suitable for terminally differentiated cell types such as macrophages or human umbilical vein endothelial cells, where it and the closely related CyTRAKOrange have been demonstrated in long-term experiments. If the cell line used shows no toxic effects during use, then the 18 h experiment can be performed continuously in the presence of DRAQ5.

5. Q: When using DRAQ5 and DRAQ7, what are the differences between them and how to choose?

A: Choose DRAQ5 for DNA analysis of live cells, long-term monitoring of cell status, or deep tissue imaging; choose DRAQ7 for assessing cell viability, detecting apoptosis, or distinguishing live/dead cell populations in samples. The differences between the two are as follows:

Comparison Index	DRAQ5	DRAQ7

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Cell Membrane Permeability	Permeable	Impermeable
Staining Target	Nuclei of all live and fixed cells	Nuclei of only dead, apoptotic or permeabilized cells
Binding Mode	Stoichiometric binding to DNA; fluorescence intensity reflects DNA content	Specific binding to dsDNA with rapid staining
Applicable Scenarios	Live cell DNA content analysis, cell cycle research, long-term live cell imaging experiments, deep tissue/tumor tissue imaging	Cell viability assessment, apoptosis detection, in vitro toxicity testing of drugs/compounds, flow cytometry experiments for distinguishing live/dead cells

Specifications

Attribute	Value
Synonyms	Ready-to-use DRAQ5 Live Cell DNA Dye
Specifications & Purity	BioReagent,ready-to-use,Biological Stain,for fluorescence analysis,for microscopy,sterile,5 mM
Stability And Storage	Store at 2-8°C long term (36 months). Store in the dark.
Storage Conditions	Store at 2-8°C,Protected from light
Shipped In	Wet ice This product requires cold chain shipping. Ground and other economy services are not available.

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