INSTRUCTIONS

DRAQ5™ Fluorescent Probe

2333.0

Number	Description
62254	**DRAQ5 Fluorescent Probe,** 5mM, 50µL, sufficient for ~4 × 96 wells
62251	**DRAQ5 Fluorescent Probe,** 5mM, 200µL, sufficient for ~16 × 96 wells
62252	**DRAQ5 Fluorescent Probe,** 5mM, 1mL, sufficient for ~80 × 96 wells
	Molecular Weight: ~413 Da
	Ex/Em Wavelength: 646/697nm

Storage: Store at 4°C protected from light. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific DRAQ5 Fluorescent Probe is a far-red DNA stain for use in live or fixed cells. Because of its far-red excitation and emission, the DRAQ5 Fluorescent Probe can be multiplexed with many other fluorophores and is ideal for cells expressing green fluorescent protein (GFP) fusion proteins. DRAQ5 Fluorescent Probe is compatible with many existing protocols across a wide range of instrumentation platforms. This fluorescent probe is part of the Thermo Scientific Cellomics Total Solution Platform for cellular imaging.

Fluorescence cell-based assays, such as flow cytometry, in-cell ELISA, fluorescence microscopy and high-content imaging require a fluorescent label to identify individual cells. When using multiple fluorescent probes to detect different cellular targets or activities, each probe must have a fluorescent spectrum different than the other probes. The blue-fluorescent DNA-binding probes, Hoechst and DAPI are frequently used; however, these probes cannot be used when UV illumination is unavailable or other blue-emitting fluorescent probes are used. Therefore, nuclear probes that emit in a color other than blue are useful for cell identification and counting, and for determining nuclear morphology and DNA content.

The DRAQ5 Fluorescent Probe emits in the far-red region, is lipophilic and crosses cell and nuclear membranes in live and fixed cells and tissues for rapid DNA staining. This stain is water-soluble, supplied ready to use and does not require RNase, cell lysis, or a washing step, making it compatible with automation. Because DNA staining is stoichiometric, the DRAQ5 Fluorescent Probe can be used for DNA content analysis in cell proliferation studies.

Procedure for DNA Staining

Note: DRAQ5 Fluorescent Probe is usually added as the last stain in a labeling procedure because no washing is required. Alternatively, add this probe in assay medium for a live cell assay. Read the supplied material safety data sheet before handling DRAQ5 Probe.

1. Prepare phosphate-buffered saline (PBS, without sodium azide) or the appropriate culture media for the specific cells.
2. Resuspend cells in PBS or media at $\leq 4 \times 10^5$ cells/mL in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
3. Add DRAQ5 Fluorescent Probe directly as supplied according to the volumes indicated in Table 1. Add directly on top of tissue sections and adherent cells or add DRAQ5 Fluorescent Probe in fresh media.
4. Gently mix and incubate for 5-30 minutes at room temperature. DRAQ5 Fluorescent Probe staining is accelerated at 37ºC and may be reduced to 1-3 minutes. For time-lapsed assays (e.g., studying translocation of an EGFP-tagged protein) DRAQ5 Fluorescent Probe may be added to the assay media for the duration of the assay (typically 0.5-3 hours) at 1µM before adding any agonist/antagonist.

   Note: Protect cells from light during incubation if other (immuno-) fluorescent stains have been applied to the cells before the DRAQ5 Fluorescent Probe staining.

5. Cells can be analyzed directly without further treatment or washing.

Table 1. Recommended volumes of the Thermo Scientific DRAQ5 Fluorescent Probe required for various cell concentrations.

Cell Sample Preparation		Volume of Probe (as supplied) Required for the Indicated Concentration		
Number of Cells	**Volume**	**5µM**	**10µM**	**20µM**
1×10^6	2,500µL	2.5µL	5µL	10µL
4×10^5	1,000µL	1µL	2µL	4µL
2×10^5	500µL	0.5µL	1µL	2µL
1×10^5	250µL	0.25µL	0.5µL	1µL
5×10^4	125µL	0.13µL	0.25µL	0.5µL

References

Edward, R. (2009). Use of DNA-specific anthraquinone dyes to directly reveal cytoplasmic and nuclear boundaries in live and fixed cells. *Mol Cells* **27:**391-6.

Martin, R.M., *et al.* (2005). DNA labeling in living cells. *Cytometry* **67A:**45-52.

Smith, P.J., *et al*. (1999). A novel cell permeant and far red-fluorescing DNA probe, DRAQ5, for blood cell discrimination by flow cytometry. *J Immunol Methods* **229(1-2):**131-9.

Smith, P.J., *et al.* (2000). Characteristics of a novel deep red/infrared fluorescent cell-permeant DNA probe, DRAQ5, in intact human cells analyzed by flow cytometry, confocal and multiphoton microscopy. *Cytometry* **40(4):**280-91.

Swerts, K., *et al*. (2007). DRAQ5: Improved flow cytometric DNA content analysis and minimal residual disease detection in childhood malignancies. *Clin Chim Acta* **379:**154-157.

Wiltshire, M., *et al.* (2000). A novel deep red/low infrared fluorescent flow cytometric probe, DRAQ5NO™, for the discrimination of intact nucleated cells in apoptotic cell populations. *Cytometry* **39(3):**217-23.

Yuan, C.M., *et al*. (2004). DRAQ5-based DNA content analysis of hematolymphoid cell subpopulations discriminated by surface antigens and light scatter properties. *Cytometry* **58:**4752.