DRAQ5™ TECHNICAL DATA SHEET

PRODUCT: DRAQ5™
PRODUCT CODES: DR05500; DR10100; DR50050; DR50200; DR51000

PRESENTATION: aqueous solution.
STORAGE: store at 2-8 °C. DO NOT FREEZE

DESCRIPTION:
DRAQ5™ is a novel far-red fluorescing DNA dye used for LIVE, permeabilized and fixed cells. It binds DNA with high affinity and stoichiometry. It can be used in combination with common visible range fluors, including FITC and R-PE and is compatible with common buffers. DRAQ5™ is highly compatible with existing protocols and a broad range of cytometry and microscopy instruments.

APPLICATIONS:
- Flow Cytometry – live or fixed
  nucleated cell gating (no lyse, no wash), Cell cycle analysis (Bjornsson et al, 2008)
- Single Cell Sorting e.g. transcriptomics, GWAS (Ordoñez-Rueda et al. 2020)
- Fluorescence Microscopy - live or fixed
- CLEM, Cryo-EM/CryoChem, ChromEMT (Tsang et al., 2018; Ou et al., 2017)
- HCS & Cell-Based Assays (Edward, 2018)
  drug, RNAi, phenotypic screens, in-cell westerns

BEFORE STARTING:
Read the MSDS. Wear protective clothing, safety goggles and laboratory gloves. Check the concentration of DRAQ5™ stated on the vial label.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:
PBS or HBSS (azide-free), culture medium (CM), phenol red-free CM ("Imaging CM"), para-formaldehyde, Triton-X 100, Tween-20, antibodies, blocking solution.

NOTE: As with any cell-permeant DNA intercalating probe, DRAQ5™ may inhibit cell division in long-term assays and should be tested for any effect.

DETECTING DRAQ5™ SIGNALS: (see Fig. 1)
Flow cytometry: DRAQ5™ can be excited by blue, green, yellow and red laser sources. Detect using longpass or bandpass filters above 660 nm and into the infra-red e.g. 780/60 BP. For DNA content, select a filter above 700 nm. Exclude doublets and reduce acquisition rates.
Microscopy / HCS Imaging Platform: DRAQ5™ is optimally excited using yellow/red wavelengths. It is detected with far-red filters above 660 nm. For DNA content, segment nuclear signals before measuring and detect above 700 nm, if possible.

EXAMPLE PROTOCOLS
As no washing is required, DRAQ5™ is added last, prior to analysis. *Use 200 µl per coverslip; 100 ul per 96-MTP well, 30 ul per 384-MTP well, 10 ul per 1536-MTP well.

PROTOCOL 1:
CELL CYCLE ANALYSIS / NUCLEATED CELL GATING BY FLOW / IMAGING CYTOMETRY

1. Prepare cells for staining with DRAQ5™: resuspend cells in appropriate buffer (PBS) at a concentration of ≤4 x 10^5 / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section size.
2. Add DRAQ5™ at 10-20 µM, final concentration. (Gating nucleated cells, this may be reduced to 5-10 µM). This will be an overlay for adherent cells / tissue sections, added to the well directly or in fresh medium following a wash step.
3. Gently mix, then incubate for 15-30 minutes at room temperature. n.b. protect from light. Staining is accelerated at 37ºC.
4. Analyze without further treatment / washing. n.b. analyze live cells within 2 h. DRAQ5™ stains live, fixed, permeabilized and dead cells.

SPECTRAL CHARACTERISTICS:
Exλ_{max} 600/646 nm Emλ_{max} 697 nm

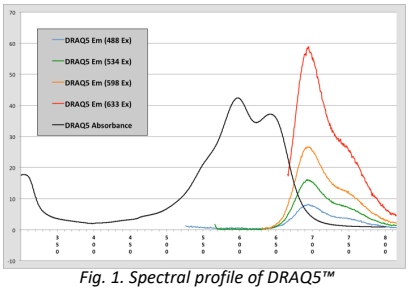

Fig. 1. Spectral profile of DRAQ5™

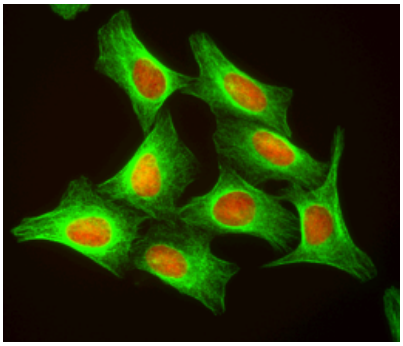
Fig. 2. DRAQ5™ (red) counterstaining of fixed U2OS cells. AlexaFluor 488 antibody to β-tubulin (green).

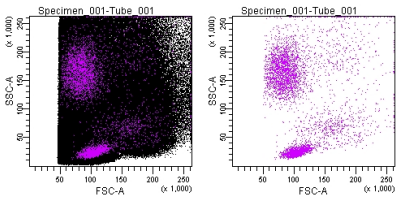

Fig.3 No lyse, no wash gating of nucleated cells from whole bone marrow gating on DRAQ5™ signal.

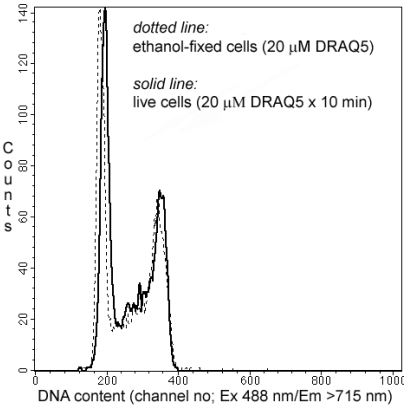

Fig. 4. DNA content analysis in live or fixed cells

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PROTOCOL 2:
LIVE CELL COUNTERSTAINING FOR HCS IMAGING PLATFORM OR FLUORESCENCE MICROSCOPY

1. Wash and aspirate the slide or well.
2. Overlay cells with DRAQ5™ - final concentration 5 µM, in Imaging CM. See guideline volumes above*
   Incubate for 15-30 minutes at room temperature. n.b. protect from light. Staining is accelerated at 37ºC.
   Note: For time-lapsed assays (e.g. studying translocation of an EGFP tagged protein) DRAQ5™ may be added to the assay medium (Imaging CM: phenol red-free to avoid background interfering fluorescence) for the duration of the assay (typically 0.5 - 3 hr.) at 1 µM prior to any agonist / antagonist addition.
3. Analyze without further treatment / washing. DRAQ5™ stains intact, live, permeabilized and dead cells. False colour DRAQ5 images red for simplicity.

PROTOCOL 3:
FIXED CELL COUNTERSTAINING FOR HCS IMAGING PLATFORM OR FLUORESCENCE MICROSCOPY

A. SEPARATE FIXATIVE & COUNTERSTAIN (e.g. when external (immuno-)fluorescent stains are applied):
1. Prepare separate working solutions of 4% formaldehyde (FA) and 5 µM DRAQ5™ in PBS.
2. Overlay slide or well with 4% FA. Incubate for 15-30 minutes at room temperature (RT) / 37ºC.
3. Gently aspirate FA, and wash with PBS. Perform any permeabilization, (immuno-)staining and blocking steps.
4. Wash with PBS to remove any residual Triton X-100, if used, and aspirate the sample. Overlay cells with DRAQ5™*.
5. Incubate for 10-20 minutes at RT. n.b. protect from light.
6. Analyze without further treatment / washing. False colour DRAQ5™ images red for simplicity.

B. COMBINED FIXATIVE & COUNTERSTAIN (e.g. when expressed fluorescent protein is the only analyte):
1. Prepare separate working solutions of 8% formaldehyde (FA) and 10 µM DRAQ5 in PBS.
2. Overlay the slide or well with equal 0.5 volumes* of FA and DRAQ5™ solutions. **Alternatively**, pre-mix DRAQ5™ and FA working solutions to overlay. See guideline volumes above*
3. Follow steps 5 & 6 from Protocol 3A.

PROTOCOL 4:
CELL ENUMERATION FOR "IN-CELL WESTERNS"

1. Seed cells at 5-6,000 cells per 96-well microtiterplate and treat with inhibitors, etc.
2. Fix cells: add 0.5 volumes of 12% formaldehyde (final concentration 4%). Incubate for 1 h. at room temperature (RT).
3. Wash the cells with PBS. Repeat twice. Aspirate.
4. Permeabilize the cells with PBS/0.1% Triton X-100. Repeat twice. Aspirate.
5. Apply blocking solution. Incubate for 2 h. at RT. Aspirate.
6. Apply unlabeled 1º antibody, in blocking solution. Incubate for 2 h. at RT. Aspirate.
7. Wash with PBS/0.1% Tween-20. Repeat x2. Aspirate.
8. Add diluted labeled 2º antibody and DRAQ5™ to 5 µM in PBS/0.5% Tween-20. Incubate for 1 h. at RT. Protect from light.
9. Repeat step 7 once.
10. Measure on a fluorescence plate reader against a standard curve from known cell numbers.

KEY REFERENCES:

Smith, P.J. et al. (2000) Cytometry 40: 280-291
Martin, R.M. et al. (2005) Cytometry A 67: 45-52
Bjornsson, S. et al. (2008) Cytometry B 74: 91-103
Ou, H.D. et al. (2017) Science 357: eaag0025
Tsang, T.K. et al. (2018) eLife7: e35524.
Edward, R. (2018) in *High Content Screening* (pp. 3-19), Humana Press, NY
Ordoñez-Rueda, D. et al. (2020) Cytometry A 97(2):156-67.

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