DRAQ5™

Vinci-Biochem®

Rapide soluzioni per la Ricerca Avanzata

rapid far-red emitting fluorescent DNA dye used for LIVE, permeabilized and fixed cell analysis

DRAQ5 (5mM)		
50µl	DR50050	250 Imaging Assays (slide); 100 Flow Cytometry Assays
200 µl	DR50200	1000 Imaging Assays (slide); 6600 HCS Assays (384-MTP); 400 Flow Cytometry Assays
1ml	DR51000	33000 HCS Assays (384-MTP)

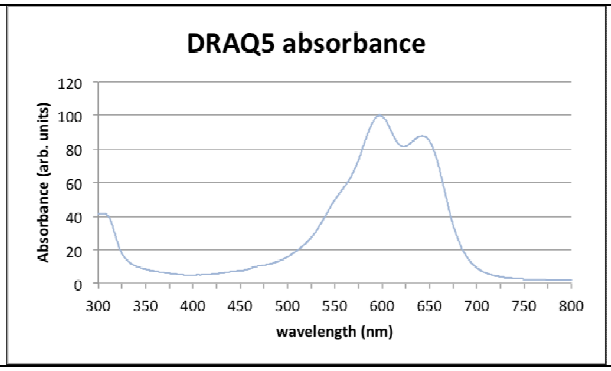
FEATURES AND BENEFITS

- Rapid staining of dsDNA/nuclei of LIVE or fixed cells
- Easy to use - no lyse, no wash, no RNase needed
- Ideal for use with GFP & FITC labels - DRAQ5 fluoresces in the far-red region
- Cost-effective analysis
- Fully compatible with CyGel™ thermoreversible gel BOS-CS20500

Background information: DRAQ5™ is a novel A-T intercalating anthraquinone. It has been chemically designed to enter the membranes of living cells, target dsDNA very specifically and contains a far-red fluorescing chromophore. DRAQ5™ is supplied in aqueous solution and is incredibly stable. It should be stored in stock solution at +4ºC. **Major Applications**. DRAQ5™ has many applications and is highly compatible with existing protocols across a wide range of instrumentation platforms:

- nucleated cell gating in flow cytometry
- cell cycle and ploidy analysis / proliferation index
- nuclear counterstain and nuclear:cytoplasm segmentation in HCS

DRAQ5™ SPECTRAL PROFILE



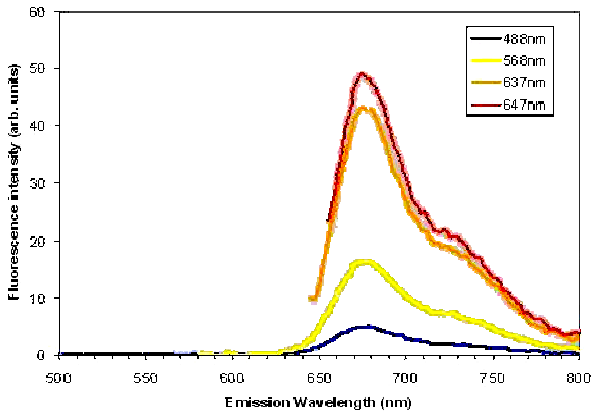
Excitation:

- Exλmax 598nm / 646 nm
- Useful excitation can be achieved at sub-optimal wavelengths from the 488 nm (flow cytometry only), 568 nm and 633 nm lines, in addition to 647nm.
- Importantly, there is no useful excitation by UV/violet light wavelengths, avoiding compound-related quenching and “white-out”

Emission:

- Emλmax 681 nm, in solution & 697 nm intercalated with dsDNA.
- There is no fluorescence enhancement on binding
- The useful fluorescence emission is from 665 nm into the low infra-red. There is minimal overlap with the visible range e.g. GFP/DsRed and FITC/PE.

The graph shows standard fluorescence emission spectra for DRAQ5™ in aqueous solution for a range of different excitation wavelengths. DRAQ5™ can be usefully excited by 488nm sources in flow cytometry systems. In these circumstances, we recommend that a long band pass filter be used - to capture as many emitted photons as possible.



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Instrument Settings:
Suggested optimal emission filters include Cy5 (not recommended

- for cell cycle analysis), 695LP, 730/50BP or 780/60BP.
- No compensation is needed with common FITC/PE antibody pairs in flow cytometry experiments.
- DRAQ5 allows multi-line imaging/cytometry when combined with UV & vis. range fluorochromes:
  up to 4 colours in multi-colour microscopy
  up to 6 colours using a 2-colour flow cytometer
- Photobleaching is extremely low allowing detection of weakly expressed reporter signals without loss of counterstain detail.
- Due to the excellent signal:noise there is no need to wash out DRAQ5 prior to imaging reducing pipetting steps and unnecessary sample processing.

DRAQ5™ EXAMPLE PROTOCOLS

Imaging

Cell staining with DRAQ5 for DNA cell cycle analysis

Last Updated: 03/09/2012

CELL STAINING WITH DRAQ5™ FOR DNA CELL CYCLE ANALYSIS BY FLOW CYTOMETRY OR BY CELL IMAGING -
Reagents required:

- DRAQ5™
- Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium

1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. Since no washing step is required, DRAQ5 will usually be the final staining procedure, after any cell treatment or labelling, prior to analysis.
3. Prepare cells for staining with DRAQ5™. Resuspend cells in appropriate buffer such as PBS at a concentration of ≤4 x 105 / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
4. Add DRAQ5™ directly as supplied following the 10 µM or 20 µM pipetting volumes in table 1. (For simple flow cytometric gating of nucleated cells the concentration of DRAQ5™ may be reduced). This will be as an overlay for adherent cells / tissue sections, added to the chamber/well liquid directly or in fresh medium following a wash step.
5. Gently mix and then incubate for 5-30 minutes at room temperature. nb: Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, prior to the DRAQ5™labelling, and which may otherwise suffer photo-bleaching. DRAQ5™ staining is accelerated at 37ºC and maybe reduced to 1-3 min. DRAQ5™ stains intact, live, fixed, permeabilized and dead cells.
6. Cells can be analysed directly without further treatment or washing.

It is also important to consider the combinations of fluorochromes and filters for the experiment:
EXCITATION: DRAQ5™ may be excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Exλ_{max} 646 nm). Despite low absorbance at 488 nm this excitation may offer optimal CVs for flow cytometric cell cycle analysis whilst allowing convenient combination with FITC and R-PE conjugates and EGFP.
EMISSION: this starts at 665 nm (Emλ_{max} 681 nm / 697 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP. For cell cycle analysis it is recommended to choose a filter (such as 715 LP) which excludes a significant proportion of signal from the small fraction of unbound DRAQ5™.

Table 1: Ready reckoner for volumes of DRAQ5™ (5mM) required for various cell concentrations:

Cell sample preparation:		VOLUME OF DRAQ5™ (AS SUPPLIED) REQUIRED FOR A CONCENTRATION OF:		
No. of cells:	**in volume:**	**5 µM**	**10 µM**	**20 µM**
1 x 10^6	2500 µl	2.5 µl	5 µl	10 µl
4 x 10^5	1000 µl	1 µl	2 µl	4 µl
2 x 10^5	500 µl	0.5 µl	1 µl	2 µl
1 x 10^5	250 µl	0.25 µl	0.5 µl	1 µl
5 x 10^4	125 µl	0.13 µl	0.25 µl	0.5 µl

LIVE cell staining with DRAQ5™ for nuclear visualisation

Last Updated: 03/09/2012

LIVE CELL STAINING WITH DRAQ5™ FOR NUCLEAR VISUALIZATION BY HCS IMAGING PLATFORM OR LASER SCANNING CONFOCAL / EPIFLUORESCENCE MICROSCOPY
Reagents required:

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- DRAQ5™
- Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium

1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. DRAQ5™ is usually added as the last stain in a labelling procedure since no washing is required or conveniently in assay medium for a live cell assay.
3. Prepare cells for staining with DRAQ5™. Resuspend cells in appropriate buffer such as PBS at a concentration of ≤4 x 105 / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
4. Add DRAQ5™ directly as supplied following the 5 µM or 10 µM pipetting volumes in table 1. This will be as an overlay for adherent cells / tissue sections, added to the chamber/well liquid directly or in fresh medium following a wash step.
5. Gently mix and then incubate for 5-30 minutes at room temperature. For time-lapsed assays (e.g. studying translocation of an EGFP tagged protein) DRAQ5™ may be added to the assay medium for the duration of the assay (typically 0.5 - 3 hr.) at 1 µM prior to any agonist / antagonist addition.

nb: Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, prior to the DRAQ5™ labelling, and which may otherwise suffer photo- bleaching. DRAQ5™ staining is accelerated at 37ºC and maybe reduced to 1-3 min. DRAQ5™ stains intact, live, permeabilized and dead cells. Cells can be analysed directly without further treatment or washing. It is also important to consider the combinations of fluorochromes and filters for the experiment:
EXCITATION: DRAQ5™ may be sub-optimally excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Exλ_{max} 646 nm). Typically, for cell imaging, excitation is typically performed with either 561 nm, 633 nm or 647 nm wavelengths.
EMISSION: Emλ_{max} 681 nm / 697 nm intercalated to dsDNA. Suitable filters include 695LP, 715LP or 780 LP. DRAQ5™ has no spectral emission overlap with FITC, R-PE, GFP and many other fluorescing proteins allowing image acquisition in one scan.

Fixed cell/tissue staining with DRAQ5 for nuclear visualisation

Last Updated: 03/09/2012

FIXED CELL/TISSUE STAINING WITH DRAQ5™ FOR NUCLEAR VISUALIZATION BY HCS IMAGING PLATFORM OR LASER SCANNING CONFOCAL / EPIFLUORESCENCE MICROSCOPY

Reagents required:
- DRAQ5™
- Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium
- Paraformaldehyde

A. **SEPARATE FIXATION AND COUNTERSTAINING STEPS** (typically for cells / tissues where one or more external (immuno-) fluorescent stains will be applied):
1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. DRAQ5 is usually added last in a procedure as no washing is required.
3. Prepare the sample for fixation and subsequent staining with DRAQ5.
4. Prepare separate working solutions of 4% formaldehyde and 5 µM DRAQ5 in PBS, pipetting 1 µl of DRAQ5, as supplied, into 1000 µl of PBS.
5. Overlay the slide or chamber/well with the 4% formaldehyde solution. Incubate for 15-30 minutes at room temperature / 37ºC.
6. Gently aspirate the formaldehyde solution, and wash with PBS.
7. Perform any necessary permeabilization, (immuno-)staining and blocking steps.
8. Overlay the washed, aspirated sample with 5µM DRAQ5 solution. Incubate for 15-30 minutes at room temperature / 37ºC, in the dark.

NOTE: Protect from the light during these incubation periods if other (immuno-) fluorescent stains have been applied to the samples, which may otherwise suffer photo-bleaching. Samples can be analysed directly without further treatment or washing.
DRAQ5™ staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type.
DRAQ5™ stains all nucleated cells.
It is also important to consider the combinations of fluorochromes and filters:
EXCITATION: DRAQ5™ may be sub-optimally excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Exλ_{max} 646 nm). Typically, for cell imaging, excitation is typically performed with either 561 nm, 633 nm or 647 nm wavelengths.
EMISSION: Emλ_{max} 681 nm / 697 nm intercalated to dsDNA. Suitable filters include 695LP, 715LP or 780 LP. DRAQ5™ has no spectral emission overlap with FITC, R-PE, GFP and many other fluorescing proteins allowing image acquisition in one scan.
It should be possible to differentially segment the nucleus and cytoplasm.

B. FOR A COMBINED FIXATION AND COUNTER-STAINING STEP (typically for cells / tissues expressing an endogenous fluorescent protein as the only analyte e.g. translocation of a GFP-tagged transcription factor), simplifying the protocol:
1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. DRAQ5™ is usually added last in a labeling as washing is not required.
3. Prepare the sample for fixation and subsequent staining with DRAQ5™.
4. Prepare separate working solutions of 8% formaldehyde (FA)and 10 µM DRAQ5 in PBS, pipetting 2 µl of DRAQ5, as supplied, into 1000 ul of PBS.
5. Overlay the slide or chamber/well with equal volumes of formaldehyde and DRAQ5 solutions. Alternatively, prepare a pre-mix of the DRAQ5 and FA working solutions to simplify and speed the workflow for multiple samples. Overlay the cells with this mixture. Incubate for 15-30 minutes at room temperature / 37ºC, in the dark.

NOTE: Protect from light during the incubation period if other (immuno-) fluorescent stains are present, which may otherwise suffer photo-bleaching. Samples can be analysed directly without further treatment or washing. DRAQ5™ staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type. DRAQ5™ stains all nucleated cells. It is important to consider

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combinations of fluorochromes / filters for the experiment:
EXCITATION: DRAQ5™ may be sub-optimally excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm ($Ex\lambda_{max}$ 646 nm). Typically, for cell imaging, excitation is typically performed with either 561 nm, 633 nm or 647 nm wavelengths.
EMISSION: $Em\lambda_{max}$ 681 nm / 697 nm intercalated to dsDNA. Suitable filters include 695LP, 715LP or 780 LP. DRAQ5™ has no spectral emission overlap with FITC, R-PE, GFP and many other fluorescing proteins allowing image acquisition in one scan.
It should be possible to differentially segment the nucleus and cytoplasm.

Cytometry

Cell staining with DRAQ5 for DNA cell cycle analysis

Last Updated: 03/09/2012

CELL STAINING WITH DRAQ5™ FOR DNA CELL CYCLE ANALYSIS BY FLOW CYTOMETRY OR BY CELL IMAGING
Reagents required:

- DRAQ5™
- Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium

1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. Since no washing step is required, DRAQ5 will usually be the final staining procedure, after any cell treatment or labelling, prior to analysis.
3. Prepare cells for staining with DRAQ5™. Resuspend cells in appropriate buffer such as PBS at a concentration of ≤4 x 105 / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
4. Add DRAQ5™ directly as supplied following the 10 µM or 20 µM pipetting volumes in table 1. (For simple flow cytometric gating of nucleated cells the concentration of DRAQ5™ may be reduced). This will be as an overlay for adherent cells / tissue sections, added to the chamber/well liquid directly or in fresh medium following a wash step.
5. Gently mix and then incubate for 5-30 minutes at room temperature.

nb: Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, prior to the DRAQ5™labelling, and which may otherwise suffer photo-bleaching.
DRAQ5™ staining is accelerated at 37ºC and maybe reduced to 1-3 min. DRAQ5™ stains intact, live, fixed, permeabilized and dead cells. Cells can be analysed directly without further treatment or washing.
It is also important to consider the combinations of fluorochromes and filters for the experiment:
EXCITATION: DRAQ5™ may be excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Exλmax 646 nm). Despite low absorbance at 488 nm this excitation may offer optimal CVs for flow cytometric cell cycle analysis whilst allowing convenient combination with FITC and R-PE conjugates and EGFP.
EMISSION: this starts at 665 nm (Emλmax 681 nm / 697 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP. For cell cycle analysis it is recommended to choose a filter (such as 715 LP) which excludes a significant proportion of signal from the small fraction of unbound DRAQ5™.

DRAQ5™ is intended for research purposes only.

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