

PRODUCT: DRAQfx™ FIX & GO
PRODUCT CODES: DFX2596, DFX7596

PRESENTATION: blue aqueous solution
STORAGE: store at 15-30 °C. DO NOT FREEZE

DESCRIPTION:

DRAQfx™ is a novel far-red fluorescing counterstain for fixed cells that binds DNA with high affinity. It can be used with UV-excited and vis. range fluors, including FITC and R-PE and is compatible with common buffers. DRAQfx™ is highly compatible with existing protocols and a broad range of fluorescence microscopes.

DRAQfx™ FIX & GO is the dropper bottle, ready-to-use product that you can keep beside your slide and multi-well preparation area.

APPLICATION:

- Immunofluorescence (IF) Microscopy – fixed cells and fixed tissue sections

BEFORE STARTING:

Read the SDS. Wear protective clothing, safety goggles and laboratory gloves.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:

Phosphate-Buffered Saline (PBS, without azide), plastic-ware, paraformaldehyde or other fixative, Triton-X 100, Tween-20, antibodies, blocking solution.

DETECTION OF DRAQfx™ SIGNALS: (see Fig. 1)

Fluorescence Microscope / HCS Imaging Platform: DRAQfx™ is optimally excited using orange and red wavelengths. It is detected with far-red filters above 670 nm. For DNA content, the nuclear signal should be segmented before measuring and detected above 700 nm, if possible.

EXAMPLE PROTOCOL

No washing is required. DRAQfx™ is diluted and the resultant solution is added last, prior to analysis. *Use 200 µl per coverslip; 100 µl per 96-MTP well, 30 µl per 384-MTP well, 10 µl per 1536-MTP well.

PROTOCOL:

FIXED CELL COUNTERSTAINING FOR AN HCS IMAGING PLATFORM OR FLUORESCENCE MICROSCOPE

A. SEPARATE FIXATIVE & COUNTERSTAIN (e.g. when external (immuno-)fluorescent stains are applied):

1. Prepare a working solution of 4% formaldehyde (FA).
2. Put 2 drops DRAQfx™ FIX & GO into each 1 ml of PBS. Invert to mix.
3. Overlay sample with 4% FA. Incubate for 15-30 min. at R.T. / 37°C.
4. Gently aspirate FA, and wash with PBS.
5. Perform any permeabilization, (immuno-)staining and blocking steps.
6. Wash and aspirate the sample. Overlay cells with DRAQfx™ counterstain solution*. Incubate for 10-20 minutes at R.T. n.b. protect from light.
7. Aspirate and overlay mountant and coverslip as required. Analyse and false colour DRAQfx™ images in red for simplicity.

B. COMBINED FIXATIVE & COUNTERSTAIN (e.g. fluorescent protein is sole analyte):

1. Prepare a working solution of 4% formaldehyde (FA).
2. Put 2 drops DRAQfx™ FIX & GO into each 1 ml of 4% FA*. Invert to mix.
3. Overlay sample with 4% FA/DRAQfx™. Incubate for 10-20 minutes at R.T. n.b. protect from light.
4. Aspirate and overlay mountant and coverslip as required. Analyse and false colour DRAQfx™ images in red for simplicity.

SPECTRAL CHARACTERISTICS:
 Exλ_{max} 601/648 nm Emλ_{max} 695 nm

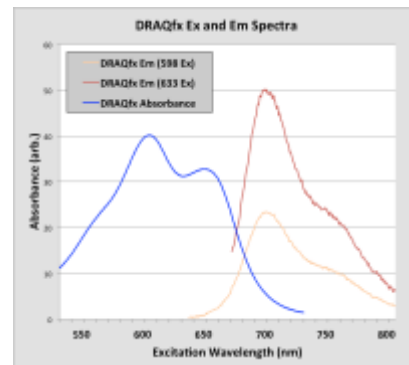


Fig. 1. Spectral profile of DRAQfx™

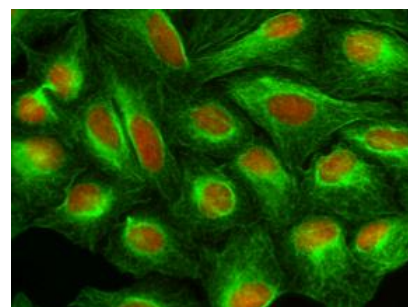


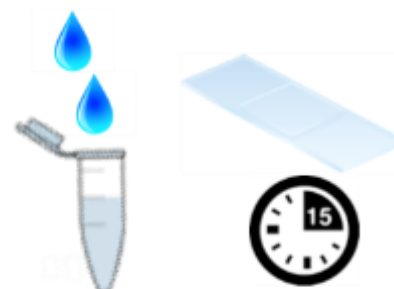
Fig. 2. DRAQfx™ (red) counterstaining of fixed U2OS cells. AlexaFluor 488 antibody to β-tubulin (green).



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QUICK PROTOCOL

1. Put 2 drops of DRAQfx™ FIX & GO into 1 ml PBS. Invert to mix.
2. Apply 100-200 µl per sample on a slide
3. Incubate for 15-20 min. at R.T.



Imaging:

- Excitation: 594 nm, 635 nm or 647 nm
- Detection: 'Cy5' or any channel between 670 and 750 nm

Handy hints:

- The active dropper bottle can be kept in the slide prep' area.
- Annotate a dropper bottle on use, copying to the pack label.
- Use diluted counterstain solution the same day.
- Store unopened dropper bottles refrigerated.

MORE INFORMATION:

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