SYTOX™ Deep Red Nucleic Acid Stain

For fixed/dead cells

Catalog Numbers S11380, S11381

Pub. No. MAN0018563 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

SYTOX™ Deep Red Nucleic Acid Stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes but does not cross the membranes of live cells. Since SYTOX™ Deep Red dye stains nucleic acid, it is particularly useful in immunocytochemistry (ICC), immunohistochemistry (IHC), or immunofluorescence (IF), experiments, where nuclei of fixed cells need to be stained. After a brief incubation with the stain, the nucleic acid of dead or fixed cells fluoresce with a deep red/far red that is detectable with a Cy5/deep red standard filter set or laser configuration (Figure 2). For flexibility in experimental setup, SYTOX™ dyes for imaging nucleus in dead/fixed cells are available in blue, green, orange and deep red colors (see "Spectral properties of SYTOX™ dyes" on page 2).

SYTOX™ Deep Red Nucleic Acid Stain can be mulitplexed with blue, green, orange, red and near IR fluorophores, when compatible fluorescence filter/laser configurations are used. The stain increases in fluorescence with increasing concentrations of dsDNA (Figure 2), but does not show much affinity for RNA or ssDNA (data not shown). SYTOX™ Deep Red Nucleic Acid Stain has a bright initial signal, and excellent photo stability in a typical imaging experiment (Figure 3). These properties make the stain ideal as a simple and quantitative single-step dead/fixed cell nucleus labeling dye for use with fluorescence microscopes, fluorimeters, fluorescence microplate readers, and flow cytometers. The stain had been successfully used on monolayer cells (Figure 4), thin tissue sections (Figure 5), thick tissue sections (Figure 6), 3D cell cultures/spheroids (Figure 7), bacteria (Figure 8), and plants (Figure 9).

Contents and storage

Each vial contains enough reagent to stain ~1,000 samples using 100 µL at 1X (0.5 µM) concentration.

Contents	Concentration	Ex/Em (nm)	Cat. No.	Amount	Storage [1]
SYTOX™ Deep Red Nucleic Acid Stain (2000X)	1 mM solution in DMSO	660/682 [2]	S11380	50 µL	• ≤ –20°C • Store upright • Protect from light
			S11381	5 × 50 µL	

[1] When stored as directed, product is stable for at least 6 months. Before refreezing, seal the vial tightly. The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation.

[2] Approximate fluorescence excitation/emission maxima in methanol when bound to DNA. Detectable with Cy5/deep red standard filter set or laser configuration.

Guidelines for staining cells

CAUTION! No data are available addressing the mutagenicity or toxicity of this reagent. Because the reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. Handle the DMSO stock solution with caution as DMSO is known to facilitate the entry of organic molecules into tissues. Solutions containing this reagent should be disposed of according to local regulations.

Determine the optimal staining concentration for each new cell line being stained. Adjust the final working concentration of the dye for each cell line being stained.

- Concentrations of 0.25 µM to 2.5 µM have been used to image HeLa, A549, U2OS, HASM, and MMM cells.
- Concentrations of 10 nM to 1 µM have been used for flow cytometry of Jurkat cells.
- Concentrations of 0.5 µM to 4 µM have been used for flow cytometry of *E. coli* cells.

Prepare 1X nucleic acid stain (0.5 µM)

1. Briefly spin down the vials, to collect all the liquid on the bottom of the vial.
2. Determine the amount of stain required, and transfer the appropriate volume to a clean tube.

   Use 100 µL of 1X stain for each sample (slide or microplate well).
3. Dilute the stock solution 2,000 fold in cell compatible buffer such as cell growth media, PBS, HBSS, or Live Cell Imaging Solution (Cat. No. A14291DJ).

   Note: This diluted staining solution should be used on the same day, and should not be stored for extended period.

Perform nuclear staining (ICC, IHC, IF)

1. Plate cells, or acquire tissue.
2. Fix the cells with 4% formaldehyde for 15 minutes and wash the cells three times with PBS, or process tissue according to standard protocol.
3. (*Optional*) Permeabilize, block, and incubate cells or tissue with antibodies using standard laboratory protocols.
4. Wash cells or tissue three times with appropriate wash buffer (e.g., PBS).
5. Add stain at 1X final concentration and stain for 30 minutes at room temperature.
6. (*Optional*) Wash cells if needed.
7. (*Optional*) Cells can be mounted using any of the standard mountant, such as ProLong™ Glass antifade mountant (Cat. No. P36980).

   Note: SYTOX™ Deep Red Nucleic Acid Stain is tested and is compatible with ProLong™ Glass, ProLong™ Diamond, ProLong™ Gold, CytoVista™ reagents, and Cytoseal™ 60 mounting media.
8. Cells can be imaged or analyzed using fluorescence microscope, or other fluorescent detecting instruments using traditional Cy5/deep red filter set, or laser configuration.

Perform nuclear staining (LIVE/DEAD Assay)

1. Treat the adherent or non-adherent cells with or without cytotoxic drugs for the specified amount of time.
2. Add the dye at 1X final concentration and incubate in a CO_2 incubator for 15 minutes at 37°C .
3. (*Optional*) Wash cells two times with PBS or similar physiological buffer to improve signal and reduce noise.
4. Image the cells by fluorescence microscopy or other fluorescence detecting instruments with traditional Cy5/deep red filter set, or laser configuration.

Spectral properties of SYTOX™ dyes

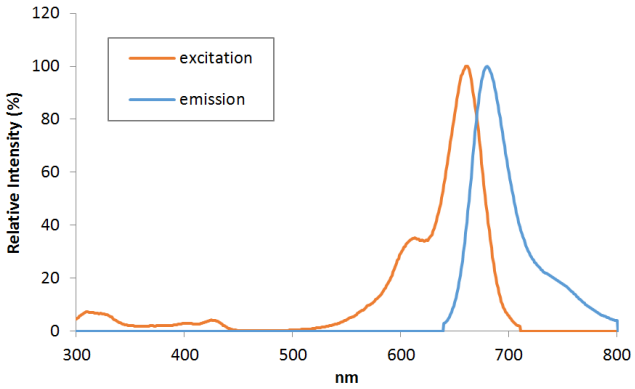

Figure 1 Fluorescence excitation and emission spectra of SYTOX™ Deep Red Nucleic Acid Stain bound to DNA
These spectra were obtained using dsDNA in Tris buffer using a Tecan™ Infinite M1000 plate reader.

Performance characteristics

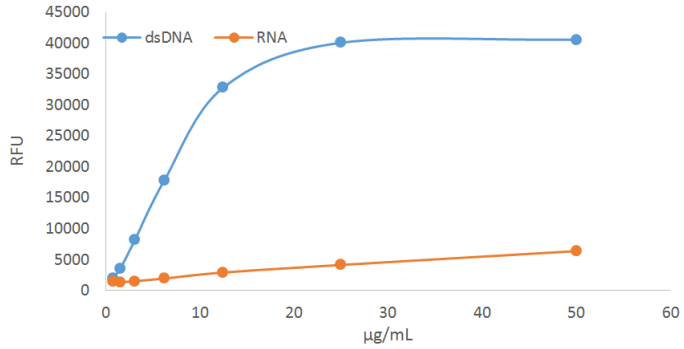

Figure 2 SYTOX™ Deep Red dsDNA vs RNA selectivity

SYTOX™ Deep Red Nucleic Acid Stain was titrated with varying concentrations of ds DNA or hairpin RNA in Tris buffer using a Tecan™ Infinite M1000 plate reader.

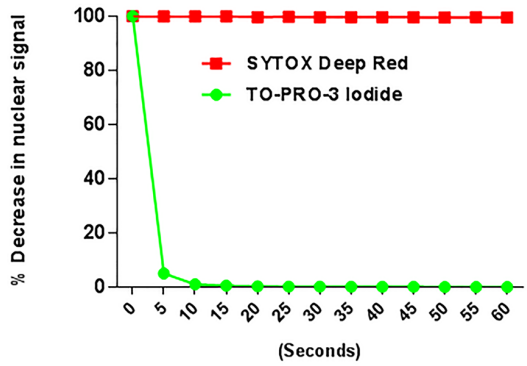

Figure 3 Photobleach curve

HeLa cells were grown on 96-well plates at a density of 5,000 cells/well. The cells were formaldehyde fixed and detergent permeabilized. The cells were then stained with either 500 nM SYTOX™ Deep Red Nucleic Acid Stain (Cat. No. S11380) or TO-PRO™-3 Iodide (Cat. No. T3605) for 30 minutes. Images were taken continuously for 1 minute at optimum exposure times on an EVOS™ FL Auto Imaging system. All the images were then quantitated using Image J.

Typical results

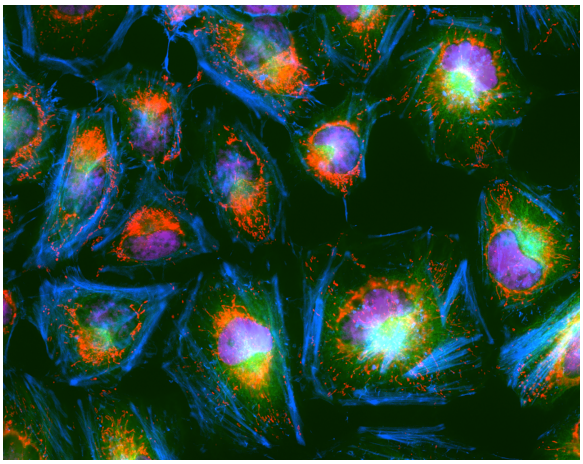

Figure 4 Staining of fixed cells

HeLa cells were grown on 96 well plates at a density of 5,000 cells/well. The cells were formaldehyde fixed and detergent permeabilized. The cells were then stained with rabbit polyclonal antibody against tubulin and mouse monoclonal antibody against complex V inhibitor protein (Cat. No. A21355) followed by Donkey Anti-Rabbit Alexa Fluor™ Plus 488 (Cat. No. A32790) and Donkey Anti-Mouse Alexa Fluor™ Plus 555 (Cat. No. A32733) respectively. The cells were then stained with SYTOX™ Deep Red Nucleic Acid Stain (Cat. No. S11380) and Alexa Fluor™ Plus 405 Phalloidin (Cat. No. A30104). Images were taken on an EVOS™ FL Auto Imaging System.

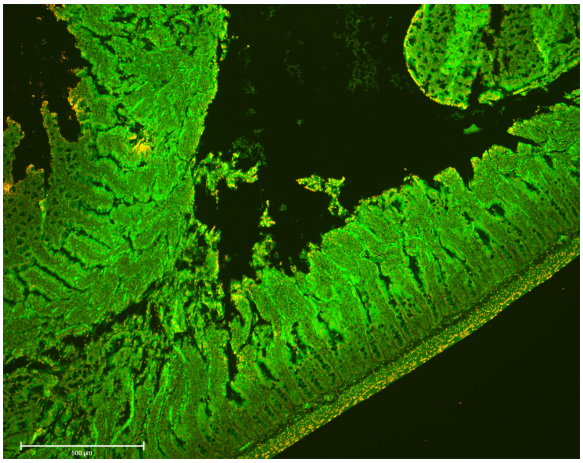

Figure 5 Staining of thin tissue sections

A formalin-fixed, paraffin embedded rat intestinal section was stained with pan actin antibody followed by Donkey Anti-Mouse Alexa Fluor™ 488 secondary antibody (Cat. No. A32766) following standard IHC protocol. The tissue sections were then stained with 500 nm SYTOX™ Deep Red Nucleic Acid Stain (Cat. No. S11380) for 30 minutes. The tissue section was then mounted with ProLong™ Glass antifade mountant (Cat. No. P36980). Images were taken using EVOS™ FL Auto Imaging System.

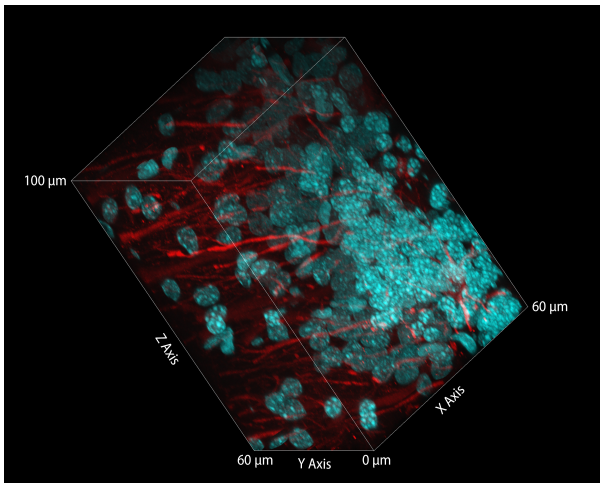

Figure 6 Staining of thick tissue sections

FFPE rat brain sections (100 µm thick), stained for tubulin (red) with Mouse Anti-Beta3-Tubulin (Cat. No. MA1-118) and Alexa Fluor™ Plus 594 Goat Anti-Mouse (Cat. No. A11032). Nuclei (cyan) were stained with SYTOX™ Deep Red Nucleic Acid Stain. Slides were mounted with ProLong™ Glass Antifade Mountant (Cat. No. P36982) and imaged with a confocal microscope using 63X oil immersion objective.

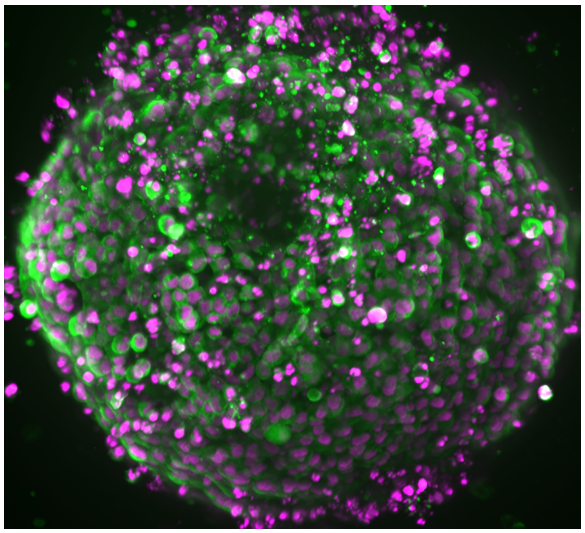

Figure 7 Staining of spheroids

HeLa cells were grown on Corning™ 384-well U-bottom spheroid plate at a density of 2,000 cells/well. The spheroids were grown for 48 hours. and then stained with rabbit polyclonal antibody in combination with Donkey Anti-Rabbit Alexa Fluor™ Plus 488 secondary antibody (Cat. No. A32790) following the standard ICC protocol. Spheroids were then stained with 500 nM SYTOX™ Deep Red Nucleic Acid Stain for 30 minutes. The spheroids were then imaged on a CellInsight™ CX7 LZR high content imaging system using confocal mode and Z sectioning. The image is a maximum intensity projection of 125 Z slices of 2 microns each.

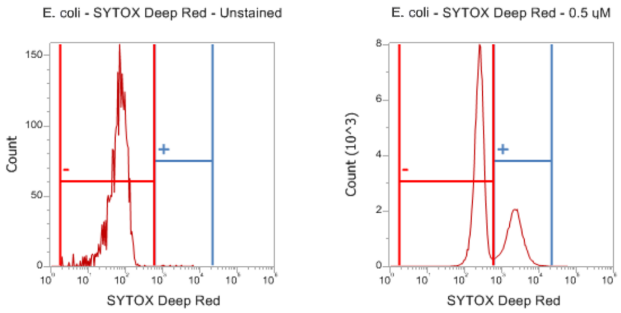

Figure 8 Staining of bacterial cells

E. coli were fixed and permeabilized for 1 hour, then stained with 500 nM SYTOX™ Deep Red Nucleic Acid Stain for 30 minutes. The data was acquired with an Attune™ NxT flow cytometer using a 638 nm laser and a 670 nm emission filter.

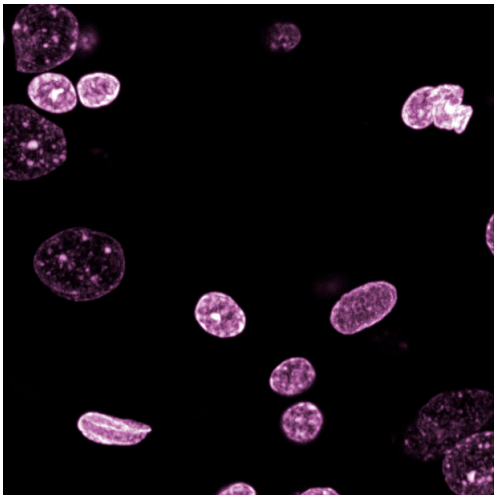

Figure 9 Staining of plant cells

A leaf section from *Zea mays L.* (sweet corn) was fixed with Image-iT™ Fixative Solution with 4% formadehyde (Cat. No. R37814), then stained with SYTOX™ Deep Red Nucleic Acid Stain. After a brief incubation with 70% ethanol, the leaf was optically cleared with Image-iT™ Plant Tissue Clearing Reagent (Cat. No. V11328) for 30 minutes, then mounted using Image-iT™ Plant Tissue Hard-set Mountant (Cat. No. V11331). Z-sections where taken with a confocal microscope at an approximate distance of 40 µm from the coverslip.

Product list

Product	Abs/Em Maxima [1]	Common filter sets	Cat. No.	Size
Cell permeable dyes for specific cellular nuclear labeling with minimum cytoplasmic background				
Hoechst 33342	350/461 nm	DAPI/UV/Blue	H3570	10 mL
			R37605	6 × 2.5 mL
Hoechst 34580	392/440 nm	Violet/CFP/DAPI	H21486	5 mg
SYTO™ 9 Green	485/495 nm	FITC/GFP/Green	S34854	100 µL
SYTO™ 82 Orange	541/560 nm	TRITC/ RFP/Orange	S11363	250 µL
SYTO™ Deep Red	652/669 nm	Cy5/Deep Red	S34900	1 vial
			S34901	5 vials
Cell impermeant dyes for specific cellular nucleus labeling with minimum cytoplasmic background				
DAPI	360/460 nm	UV/Blue/DAPI	D21490	10 mg
			R37606	6 × 2.5 mL
SYTOX™ Blue Nucleic Acid Stain	444/480 nm	Violet/CFP/DAPI	S11348	250 µL
SYTOX™ Green Nucleic Acid Stain	504/523 nm	FITC/GFP/Green	S7020	250 µL
			R37109	6 × 2.5 mL
SYTOX™ Orange Nucleic Acid Stain	547 /570 nm	RFP/TRITC	S11368	250 µL
SYTOX™ Deep Red Nucleic Acid Stain	660/682 nm	Cy5/Deep Red	S11380	50 µL
			S11381	5 × 50 µL

[1] Absorption and fluorescence emission maxima determined in the presence of DNA

Limited product warranty

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