SYTO™ Deep Red Fluorescent Nucleic Acid Stain

For live cells

Catalog Numbers S34900, S34901

Pub. No. MAN0018659 **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

SYTO™ Deep Red Nucleic Acid Stain is a cell permeable nucleic acid stain suitable for delineating the nuclei of live, dead, or fixed cells. SYTO™ Deep Red dye specifically stains cellular nuclei making it particularly useful for live cell nuclear staining, where it will stain all cells, whether they are dead or alive. It is also compatible with fixed cell workflows including immunocytochemistry (ICC), immunohistochemistry (IHC), or immunofluorescence (IF) experiments. After a brief incubation with the stain, the nucleic acid of live, 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 1).

SYTO™ Deep Red Nucleic Acid Stain increases in fluorescence with increasing concentrations of dsDNA, but does not show much response with RNA (Figure 2). The fluorescence response to dsDNA is four times the response to RNA while no difference is seen in the response with DRAQ5™ dye (Figure 3). SYTO™ Deep Red Nucleic Acid Stain has a bright initial signal, and excellent photo stability in typical imaging experiments. 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 can be mulitplexed with blue, green, orange, and red fluorophores, as well as GFP and RFP (Figure 4) when compatible fluorescence filter/laser configurations are used, and has been successfully used on monolayer cells (Figure 4), spheroids (Figure 5), or as a nuclear counter stain in ICC (Figure 6).

Contents and storage

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

Contents	Cat. No. S34900	Cat. No. S34901	Ex/Em (nm)	Storage [1]
SYTO™ Deep Red Nucleic Acid Stain	1 vial [2]	5 vials [2]	652/669 [3]	• –20°C to –5°C • Store desiccated • Protect from light
Probenecid (77 mg, lyophilized)	1 vial	1 vial	—	

[1] When stored as directed, product is stable for at least 6 months.
[2] Each vial makes 2 mM solution at 2000X concentration, when dissolved in 50 µM DMSO
[3] Approximate fluorescence excitation/emission maxima in Tris/EDTA buffer 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.
  A 1 µM final concentration of the dye was used to stain HeLa, A549, U2OS, HASM, and MMM cells.
- Probenecid treatment is optional, but it helps to retain SYTO™ Deep Red Nucleic Acid Stain in live cells for many cell lines. To minimize off-target effects on other cellular functions, we recommend using 1X Probenecid while loading and imaging with SYTO™ Deep Red Nucleic Acid Stain. Do not incubate in Probenecid for more than a few hours.
- SYTO™ Deep Red Nucleic Acid Stain is compatible with ICC workflows, but SYTOX™ Deep Red Nucleic Acid Stain (Cat. No. S11381) better suited for this application.
- For optimal result, image live cells within 1–2 hours. Longer time periods are possible, but have not been tested.

Prepare stock solutions

1. Add 50 µL of DMSO to a vial of SYTO™ Deep Red Nucleic Acid Stain to make 2000X SYTO™ Deep Red Stock Solution (2 mM).

2. Add 1 mL of live cell compatible buffer to a vial of lyophilized Probenecid powder to make 100X Probenecid Stock Solution.

Stock solutions are stable for up to 3 months when stored at –20°C.

Prepare 1X SYTO™ Working Solution

1. Dilute SYTO™ Deep Red Stock Solution 2000 times in live cell compatible buffer/growth medium to make a 1 µM SYTO™ Working Solution.

2. (*Optional*) Dilute Probenecid Stock Solution 100 times in SYTO™ Working Solution for a 1X final concentration.

Use the working solution on the same day it is prepared.

Perform nuclear staining

This procedure stains both live and dead cells.

1. Add 1X SYTO™ Working Solution to cells and incubate for 30 minutes at 37°C.

   Note: Incubation time can be increased to 1–2 hours if performing 3D cell culture staining.

2. (*Optional*) Wash cells two times with Live Cell Imaging Solution (LCIS)/HBSS to reduce background staining.

3. (*Optional*) Fix cells with 2–4% formaldehyde solution.

   Image fixed cells within 24–48 hours for best results.

4. Image the cells by fluorescence microscopy or other fluorescence detecting instruments with Cy5/deep red filter set, or 647 red laser configuration.

Spectral properties of SYTO™ Deep Red dye

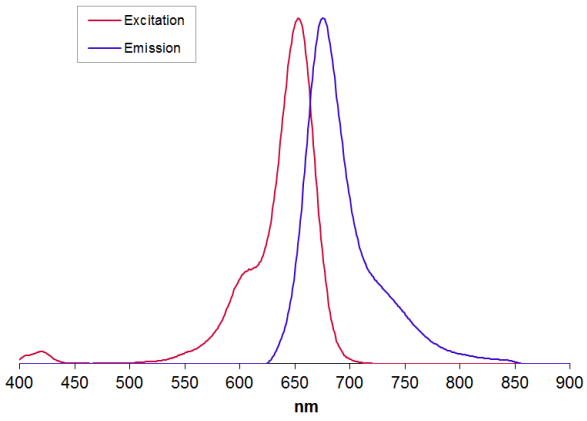

Figure 1 Fluorescence excitation and emission spectra of SYTO™ Deep Red Nucleic Acid Stain bound to DNA
These spectra were obtained using dsDNA in Tris buffer using a Tecan™ Infinite M1000 plate reader.

Performance characteristics

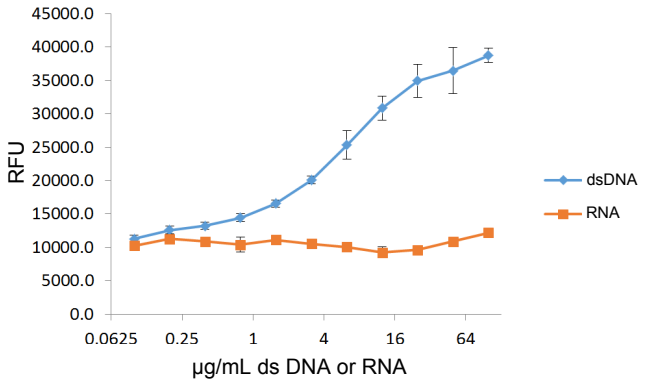

Figure 2 Fluorescence response to dsDNA or RNA

10 nM SYTO™ Deep Red fluorescence response to dsDNA or RNA at various concentrations in TA buffer detected by Tecan™ Infinite M1000 with ex/em 650/680 nm. x-axis is represented at LOG2 with error bars representing standard deviation (n:4).

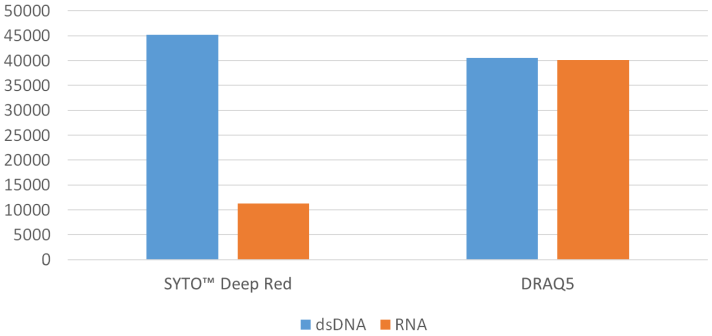

Figure 3 SYTO™ Deep Red vs DRAQ5™, fluorescence response to dsDNA vs RNA

Fluorescence response of SYTO™ Deep Red or DRAQ5™ (another deep red nucleic acid stain) measured with Tecan™ Infinite M1000 in presence of 50 µg/mL RNA or DNA. SYTO™ Deep Red shows at least four times more fluorescence in the presence of dsDNA compared to RNA. DRAQ5™ does not have an increased fluorescence response to dsDNA, and shows a similar response between RNA and DNA.

Typical results

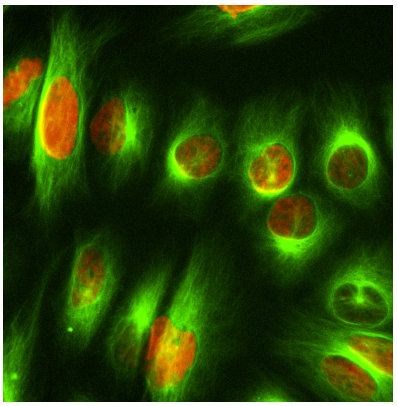

Figure 4 Dual staining image of live culture of HeLa cells

HeLa cells were grown on a Greiner 96-well plate at a density of 5,000 cells/well, then stained with 1 µM SYTO™ Deep Red Nucleic Acid Stain and 1 µM Tubulin Tracker™ Green (Cat. No. T34078) for 30 minutes. The

cells were then washed and imaged on an EVOS™ M7000 Imaging System using 40X objective.

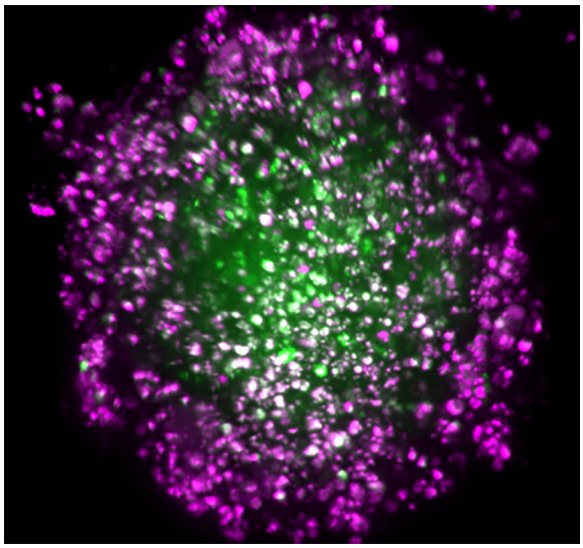

Figure 5 Staining of spheroids

HeLa cells were plated on a Nunclon™ Sphera™ U-well plate (Cat. No. 174929) at a density of 5,000 cells/well and left for 24 hours in a CO_2 incubator to form spheroids. The spheroids were treated with 40 µM niclosamide for 24 hours. The spheroids were then stained with 1 µM SYTO™ Deep Red Nucleic Acid Stain and 5 µM CellEvent™ Caspase-3/7 Green Detection Reagent (Cat. No. C10723) for 1 hour at 37°C in a CO_2 incubator. The spheroids were then washed and imaged on a CellInsight™ CX7 LZR High Content Analysis Platform (Cat. No. CX7B1112LZR). The image is a maximum intensity projection of 25 Z slices at 10 micron each.

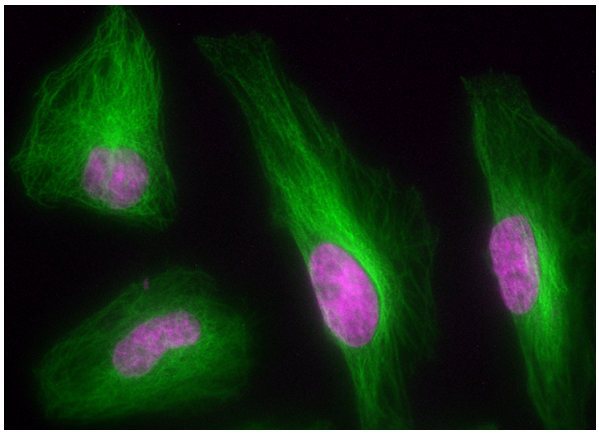

Figure 6 Multiplex image of immunocytochemistry (ICC), using HeLa cells

HeLa cells were grown on a Greiner 96-well plate at a density of 5,000 cells/well and left overnight at 37°C in a CO_2 incubator. The cells were then stained with 1 µM SYTO™ Deep Red Nucleic Acid Stain for 30 minutes. The cells were then washed, fixed and stained with anti-tubulin antibody and Alexa Fluor™ Plus 488 Donkey anti-Mouse IgG secondary antibody (Cat. No. A32766) following a standard ICC protocol. The cells were then imaged on EVOS™ M7000 Imaging System using 40X objective (Cat. No. AMF7000).

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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