Revised: June 28, 2021

Product Information

Cyanine Nucleic Acid Dyes

Dye	Cat no.	Unit size	Ex/Em (nm) with DNA	MW
Oxazole Blue (PO-PRO™-1), 1 mM in DMSO	40091	1 mL	434/457	579
Oxazole Blue Homodimer (POPO™-1), 1 mM in DMSO	40093	100 uL	433/457	1170
Oxazole Yellow (YO-PRO®-1), 1 mM in DMSO	40089	1 mL	491/506	629
Oxazole Yellow Homodimer (YOYO®-1), 1 mM in DMSO	40090	100 uL	491/508	1271
Oxazole Gold (SYBR® Gold), 10,000X in DMSO	40094	500 uL	496/539	749
Thiazole Green (SYBR® Green I), 10,000X in DMSO	40086-0.5mL	500 uL	498/522	545
	40086-1mL	1 mL		
Thiazole Orange, 10 mM in DMSO	40077	1 mL	512/533	477
TO Iodide (TO-PRO®-1), 1 mM in DMSO	40088	1 mL	515/531	645
Thiazole Orange Homodimer (TOTO®-1), 1 mM in DMSO	40079	200 uL	514/531	1303
Oxazole Red (YO-PRO®-3), 1 mM in DMSO	40105	1 mL	613/629	655
Oxazole Red Homodimer (YOYO®-3), 1 mM in DMSO	40106	200 uL	612/631	1323
Thiazole Red (TO-PRO®-3), 1 mM in DMSO	40087	1 mL	642/657	671
Thiazole Red Homodimer (TOTO®-3), 1mM in DMSO	40080	200 uL	642/661	1355

Storage and Handling

Store at 4°C or below, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

Cyanine nucleic acid stains are highly sensitive fluorescent dyes for DNA and RNA. The dyes are essentially non-fluorescent in the absence of nucleic acids but exhibit strong fluorescence when bound to nucleic acids. Biotium offers both dimeric and monomeric forms of commonly used cyanine nucleic acid stains. With the exception of Thiazole Green (SYBR® Green I), Oxazole Gold (SYBR® Gold), and Thiazole Orange which are membrane-permeant, all other dyes included in this product information sheet are membrane-impermeant. Nucleic acid dyes that are membrane-impermeant may be used for selective dead cell staining by microscopy or flow cytometry. In addition, the membrane-impermeant dyes are non-cytotoxic and may be used for long-term monitoring of viability in cell cultures.

Thiazole Green, also known as SYBR® Green I, is a widely-used nucleic acid stain for detecing double-stranded DNA (dsDNA), in agarose, polyacrylamide gels, and qPCR. Thiazole Green can also be used to detect ssDNA and RNA in denaturing agarose/formaldehyde and polyacrylamide/urea gels without any prewashing steps, although the sensitivity is lower. As a membrane-permeant dye, Thiazole Green may also be used to stain all cells in live cultures. However, Thiazole Green is also cytotoxic and not suitable for long-term cell staining. EvaGreen® Dye and EvaGreen® Plus Dye are Biotium's superior alternatives to Thiazole Green, offering less PCR inhibition and more robust signal for qPCR and high-resolution DNA melt curve analysis. Biotium also offers NucSpot® Live and Hoechst stains which are non-toxic and more suitable for long-term live cell staining (see Related Products).

Oxazole Gold (SYBR® Gold) is the most sensitive gel stain available for DNA and RNA, particularly for single-stranded, oligos, and denaturing gels. The dye exhibits >1000-fold fluorescence enhancement upon binding to nucleic acids and is 25-100 times more sensitive than ethidium bromide. It has also been used for selective staining of mitochondrial nucleoids in live cells.

For nuclear specific counterstains for live cultures in the green and far-red channels, please see our NucSpot® Live 488, NucSpot® Live 650, and RedDot™1. Biotium also offers nuclear specific NucSpot® 470, NucSpot® Far-Red, and RedDot™2, which are suitable for fixed cells and selectively stains dead cells in live cultures (see Related Products).

Dye Properties

Dye	Chemical Formula	Color and Form	Cell Permeability
Oxazole Blue	$C_{20}H_{27}I_2N_3O$	Yellow liquid	Impermeant
Oxazole Blue Homodimer	$C_{41}H_{54}I_4N_6O_2$	Yellow liquid	Impermeant
Oxazole Yellow	$C_{24}H_{29}I_2N_3O$	Orange liquid	Impermeant
Oxazole Yellow Homodimer	$C_{49}H_{58}I_4N_6O_2$	Yellow liquid	Impermeant
Oxazole Gold	$C_{32}H_{37}I_2N_3O_2$	Orange liquid	Permeant
Thiazole Green	$C_{32}H_{37}ClN_4S$	Orange liquid	Permeant
Thiazole Orange	$C_{26}H_{24}N_2O_3S_2$	Orange liquid	Permeant
TO Iodide	$C_{24}H_{29}I_2N_3S$	Orange liquid	Impermeant
Thiazole Orange Homodimer	$C_{49}H_{58}I_4N_6S_2$	Orange liquid	Impermeant
Oxazole Red	$C_{26}H_{31}I_2N_3O$	Blue liquid	Impermeant
Oxazole Red Homodimer	$C_{53}H_{62}I_4N_6O_2$	Blue liquid	Impermeant
Thiazole Red	$C_{26}H_{31}I_2N_3S$	Blue liquid	Impermeant
Thiazole Red Homodimer	$C_{53}H_{62}I_4N_6S_2$	Blue liquid	Impermeant

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qPCR Protocol for Thiazole Green (SYBR® Green I)

The following is an example protocol for qPCR using Biotium's Cheetah™ HotStart Taq (see Related Products) and Thiazole Green. Reaction conditions may require optimization for different applications.

1. Set up PCR reaction using the following final concentrations of reaction components:
   - 1X Cheetah™ Taq Polymerase Buffer
   - 2.5 mM $MgCl_2$
   - 0.1-1 uM each of primers
   - 0.2 mM each of dNTPs
   - 0.02-0.1 unit/uL Cheetah™ HotStart Taq DNA Polymerase
   - 1X Thiazole Green (SYBR® Green I)
   - Optional ROX Reference Dye (if required by your instrument)
   - dH_2O to required final reaction volume
2. Perform real-time PCR on a qPCR instrument and acquire the fluorescence signal at the annealing or extension step with the SYBR® Green or FAM channel.

DNA/RNA Gel Staining Protocol for Oxazole Gold (SYBR® Gold)

1. Before use, warm up the vial of Oxazole Gold, 10,000X in DMSO to room temperature and briefly centrifuge to collect the DMSO solution at the bottom of the vial.
2. Run gels as usual according to your standard protocol.
3. Dilute Oxazole Gold to 1X concentration using working strength electrophoresis buffer or water. For example, add 5 uL of 10,000X Oxazole Gold dye to 50 mL of TAE, TBE, or water and mix completely.

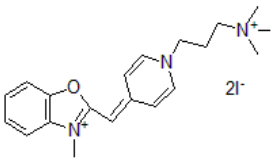
   Note: For optimal sensitivity, verify that the pH of the Oxazole Gold staining solution is between 7.0 and 8.5.
4. Carefully place the gel in a suitable container such as a polypropylene staining tray. Add a sufficient amount of Oxazole Gold staining solution to submerge the gel and protect from light.
5. Agitate the gel gently at room temperature for ~10-40 minutes.
6. Destaining is not required, although the gel can be washed in water to reduce background if necessary.

   Note: The staining solution may be stored in the dark and can be reused 3-4 times. For best results, a fresh staining solution is recommended.
7. Image gels using a blue light transiluminator or a UV transilluminator. A SYBR® Green filter is optimal for SYBR® Gold, but staining also can be imaged using an EtBr filter.

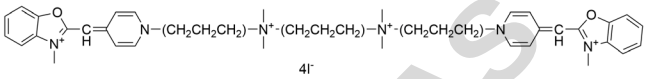
Selective staining of dead cells

1. Dilute the membrane-impermeant cyanine nucleic acid dye to a final concentration of 100 nm in complete cell culture medium or buffer of your choice.

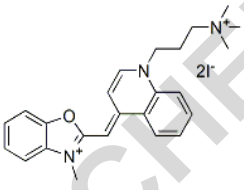
   Note: For optimal results, we recommend a titration range between 50 nm to 1 uM for different applications.
2. Incubate cells with the dye solution for 15 minutes or longer at room temperature or 37°C.
3. Analyze cells by microscopy or flow cytometry in the appropriate channel (see excitation and emission values in the product table).



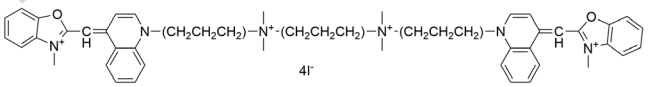
Oxazole Blue (PO-PRO™-1)
Full chemical name: Benzoxazolium, 3-methyl-2-[[1-[3-(trimethylammonio)propyl]-4(1H)-pyridinylidene]methyl]-, diiodide.
CAS number: 157199-56-9



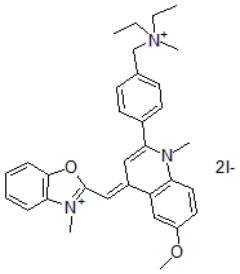
Oxazole Blue Homodimer (POPO™-1)
Full chemical name: Benzoxazolium, 2,2'-[1,3-propanediylbis[(dimethylimin io)-3,1-propanediyl-1(4H)-pyridinyl-4-ylidenemethylidyne]]bis[3-methyl]-, tetraiodide
CAS number: 169454-15-3



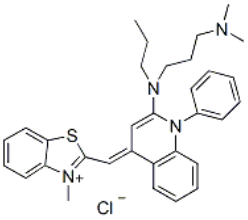
Oxazole Yellow (YO-PRO®-1)
Full chemical name: 4-[(3-Methyl-2(3H)-benzoxazolylidene)methyl]-1-[3-(trimethylammonio)propyl]-quinolinium diiodide
CAS number: 152068-09-2



Oxazole Yellow Homodimer (YOYO®-1)
CAS number: 143413-85-8

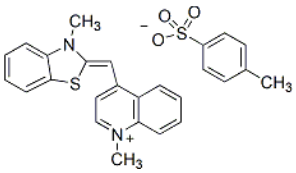


Oxazole Gold (SYBR® Gold)
CAS number: NA

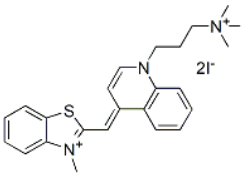


Thiazole Green (SYBR® Green I)
Full chemical name: N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine
CAS number: 163795-75-3

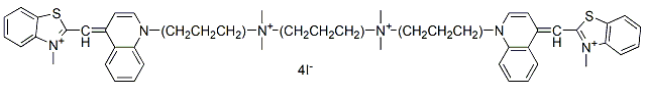
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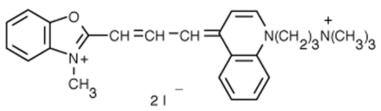
Thiazole Orange
Full chemical name: 1-Methyl-4-[(3-methyl-2(3H)-benzothiazolylidene)methyl] quinolinium p-tosylate
CAS number: 107091-89-4



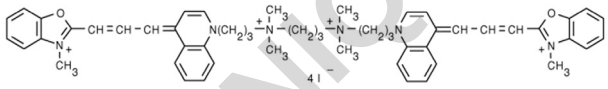
TO Iodide (TO-PRO®-1)
Full chemical name: Quinolinium, 4-[(3-methyl-2(3H)-benzothiazolylidene) methyl]-1-[3-(trimethylammonio)propyl]-, diiodide
CAS number: 157199-59-2



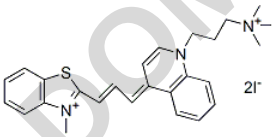
Thiazole Orange Homodimer (TOTO®-1)
Full chemical name: Quinolinium, 1-1'-[1,3-propanediylbis[(dimethyliminio)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzothiazolylidene)methyl]]-, tetraiodide
CAS number: 143413-84-7



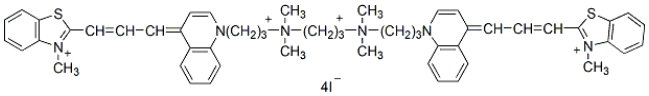
Oxazole Red (YO-PRO®-3)
CAS number: 157199-62-7



Oxazole Red Homodimer (YOYO®-3)
CAS number: 156312-20-8



Thiazole Red (TO-PRO®-3)
CAS number: NA



Thiazole Red Homodimer (TOTO®-3)
CAS number: NA

Related Products

Catalog number	Product
40083	NucSpot® 470 Nuclear Stain for dead or fixed cells
40085	NucSpot® Far-Red dead cell stain for flow cytometry
40081	NucSpot® Live 488 Nuclear Stain for live or fixed cells
40082	NucSpot® Live 650 Nuclear Stain for live or fixed cells
40060	RedDot™1 Far-Red Nuclear Stain for live cells
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
23001... 23020	EverBrite™ Mounting Medium
23008, 23009	Drop-n-Stain EverBrite™ Mounting Medium
23003... 23021	EverBrite™ Hardset Mounting Medium
23017... 23022	EverBrite TrueBlack® Hardset Mounting Medium
40037	7-AAD, 1 mg
40084	7-AAD, 1 mg/mL solution
40014	Ethidium Homodimer I, 2 mM in DMSO
40051	Ethidium Homodimer III, 1 mM in DMSO
40048	Propidium Iodide, 50 ug/mL in Buffer
31077	EvaGreen® Plus Dye, 20X in Water
31000	EvaGreen® Dye, 20X in water
29050	Cheetah™ HotStart Taq DNA Polymerase, 500 U

Please visit our website at www.biotium.com for information on our life science research products, including GelRed® and GelGreen® nucleic acid gel stains, organelle stains, Live-or-Dye™ Fixable Viability Staining Kits, and other viability dyes.

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