Cell Viability and Cell Death

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Biotium

NucView® Caspase-3 Substrates

Fluorescent Caspase-3 Detection in Living Cells

NucView® caspase-3 substrates are novel fluorescent probes that allow detection of caspase-3/7 activity in intact cells in real-time. In contrast to other fluorogenic caspase substrates or fluorescent caspase inhibitor based (FLICA) assays, NucView® substrates can be used to detect caspase-3/7 activity in cells without inhibiting apoptosis progression.

NucView® is made by attaching a nucleic acid binding dye to the caspase-3/7 substrate peptide sequence DEVD. This uncleaved substrate is unable to bind to DNA and remains non-fluorescent. Once the substrate is cleaved by caspase-3/7 in apoptotic cells, it releases the high-affinity fluorescent DNA dye, which stains the cell nucleus with bright and stable fluorescent signal.

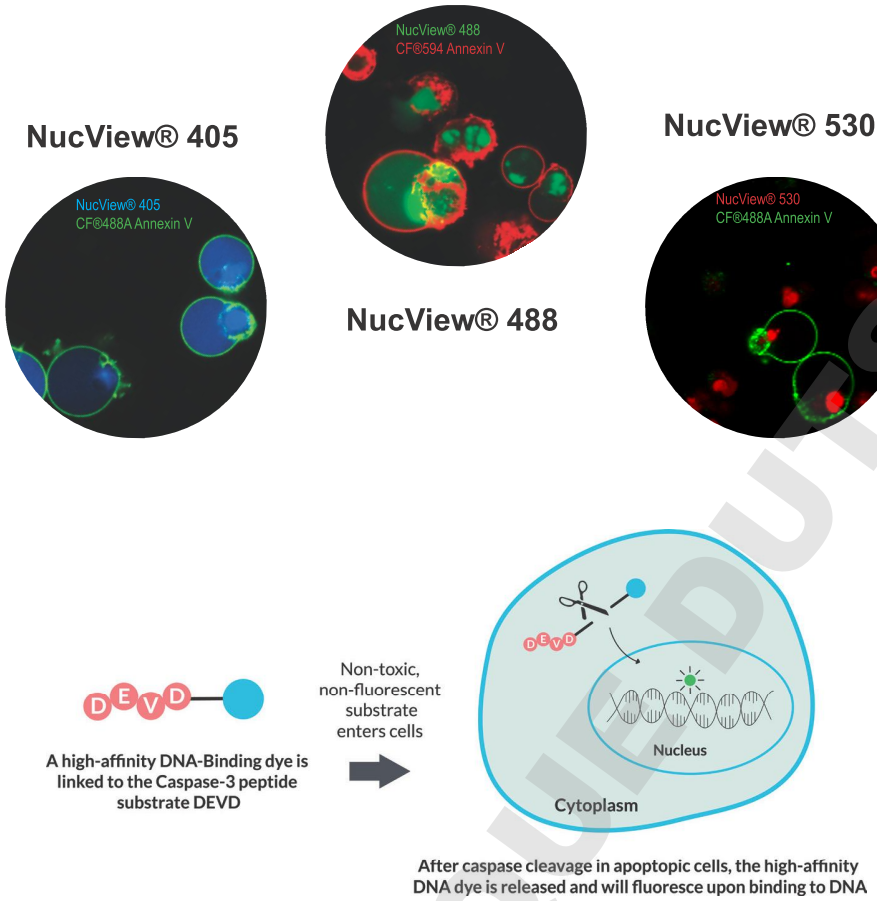


Figure 1. Principle of intracellular caspase-3/7 detection using NucView® caspase-3 substrates.

NucView® Features

- Endpoint or real-time assays
- Simple, no-wash protocol
- Non-toxic, multi-day experiments possible
- Available in 3 colors
- For flow cytometry, microscopy or live cell imaging systems
- Dual detection of caspase activity and nuclear morphology
- Formaldehyde fixable

Proven Technology

NucView® caspase detection technology has been extensively tested and validated.

- Published in over 200 scientific papers
- Validated in more than 100 cultured cell lines and 50 primary cell types

NucView® Substrates

Product	Cat. #	Size
NucView® 488 Caspase-3 Substrate, 1 mM in DMSO	10402-T	10 uL
	10402	100 uL
NucView® 488 Caspase-3 Substrate, 1 mM in PBS	10403-T	10 uL
	10403	100 uL
NucView® 405 Caspase-3 Substrate, 1 mM in DMSO	10405-T	10 uL
	10405	100 uL
NucView® 405 Caspase-3 Substrate, 1 mM in PBS	10407-T	10 uL
	10407	100 uL
NucView® 530 Caspase-3 Substrate, 1 mM in DMSO	10406-T	10 uL
	10406	100 uL
NucView® 530 Caspase-3 Substrate, 1 mM in PBS	10408-T	10 uL
	10408	100 uL

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NucView® Kits and Other Caspase Assays

NucView® Combination Staining Kits

Biotium also offers kits containing the NucView® 488 substrate together with other types of apoptosis and viability dyes for convenient multi-parameter experiments.

- Dual Apoptosis Kit: NucView® 488 + Annexin V labeled with red or far-red dyes for co-detection of two apoptotic events, caspase cleavage and phosphatidylserine (PS) translocation. For more Annexin V conjugates see page 4.
- Dual Apoptosis Kit: NucView® 488 + MitoView™ 633 for co-detection of two apoptotic events, caspase cleavage and loss of mitochondrial membrane potential. For more information on MitoView™ see page 6.
- Apoptosis/Necrosis Kit: NucView® 488 + RedDot™2 for concurrent measurement of caspase cleavage (apoptosis) and loss of membrane integrity (necrosis).

Additional Caspase Substrates

In addition to our patented NucView® technology for detecting caspase-3 activity in live cells, Biotium also offers rhodamine 110 (R110)-based assay kits for fluorescence- or absorbance-based detection of caspase-3 or caspase-8 activity in cell lysates. The HTS versions of the R110-based homogenous caspase-3 and caspase-8 assay kits are optimized for high-throughput screening by fluorescence microplate reader.

Biotium also offers a coumarin (AMC)-based blue fluorogenic substrate (Ac-DEVD-AMC) for measuring caspase-3 activity in cell lysates by fluorescence microplate reader.

Caspase Inhibitor

Ac-DEVD-CHO is a competitive inhibitor of caspase-3 for use in cultured cells or cell lysates.

Apoptosis Inducers

Staurosporine is a broad-range protein kinase inhibitor that induces apoptosis in cultured cells. It is useful as a positive control for many apoptosis assays. We also offer ionomycin, a calcium ionophore that has been shown to induce apoptosis through calpain activation.

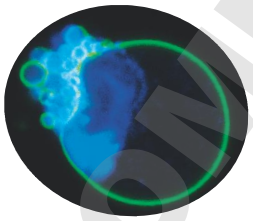


Figure 2. Apoptotic HeLa cell stained with CF®488A Annexin V (green) and NucView® 405 (cyan). See p. 4 for more information on Annexin V conjugates.

NucView® Combination Kits and Other Caspase Substrates and Inhibitors

Product	Cat. #	Size
Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V	30067	50 assays
Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®640R Annexin V	30073	50 assays
NucView® 488 and MitoView™ 633 Apoptosis Assay Kit	30062	100 assays
NucView® 488 and RedDot™2 Apoptosis & Necrosis Kit	30072	100 assays
Caspase-3 DEVD-R110 Fluorometric & Colorimetric Assay Kit	30008-1	25 assays
	30008-2	100 assays
Caspase-3 DEVD-R110 Fluorometric HTS Assay	30009-1	10 assays
	30009-2	100 assays
	30009-3	1000 assays
Caspase-8 IETD-R110 Fluorometric & Colorimetric Assay Kit	30011-1	25 assays
	30011-2	100 assays
Caspase-8 IETD-R110 Fluorometric HTS Assay	30012-1	10 assays
	30012-2	100 assays
	30012-3	1000 assays
Ac-DEVD-CHO Caspase-3 Inhibitor	10404-1	1 mg
	10404	5 mg
Ac-DEVD-AMC	10202	5 mg
Staurosporine	00025	100 ug
Ionomycin, Calcium Salt	59007	1 mg

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Annexin V Conjugates

Annexin V is a 35-36 kDa protein that has a high affinity for phosphatidylserine (PS). During apoptosis, PS is translocated from the inner to the outer leaflet of the plasma membrane, where it can be stained by fluorescent conjugates of Annexin V, for detection of apoptotic cells by flow cytometry (Fig. 1) or fluorescence microscopy. Biotium offers Annexin V conjugates and kits featuring our exceptionally bright and photostable CF® dyes. Due to their excellent brightness, our CF® Dye Annexin Conjugates can be used for multi-day, real-time imaging in cell culture without a wash step.

Annexin V staining is commonly performed in Annexin Binding Buffer, which is optimized for staining. We offer Annexin V conjugates in stable liquid format with azide for endpoint staining, or in azide-free lyophilized format for real-time cell imaging.

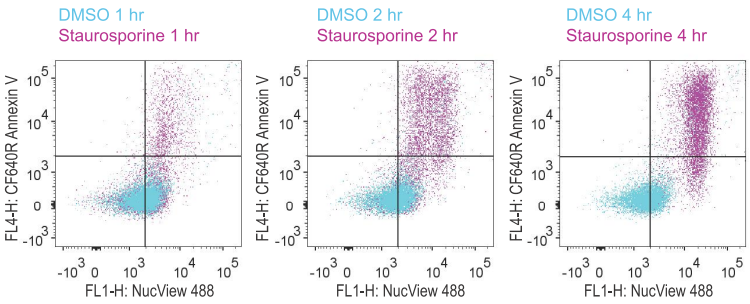


Figure 1. Jurkat cells were treated with staurosporine to induce apoptosis (pink), or with DMSO as a negative control (blue) for the times indicated, then stained for 15 minutes at room temperature with NucView® 488 Caspase-3 Substrate (FL1-H, x-axis) and CF®640R Annexin V (FL4-H, y-axis) in cell culture medium prior to analysis using a BD LSRII flow cytometer. See pp. 2-3 for more information on NucView® caspase-3 substrates.

Annexin V CF® Conjugate Features

- Featuring CF® dyes, Biotium's next-generation dyes with superior brightness & photostability
- Fast, reliable detection of apoptotic cells
- Compatible with fluorescent microscopy, live cell imaging and flow cytometry
- Available in lyophilized and preservative-free formulations for real-time or *in vivo* imaging
- Wide variety of CF® dye colors, plus biotin, R-PE, APC, and other fluorescent dyes

Annexin V Conjugates

Product	Ex/Em (nm)	Cat. #
CF®350 Annexin V, 50 ug/mL	347/448	29012
CF®405M Annexin V, 50 ug/mL	408/452	29009
CF®488 Annexin V, 50 ug/mL	490/515	29005
CF®555 Annexin V, 50 ug/mL	555/565	29004
CF®568 Annexin V, 50 ug/mL	562/583	29010
CF®594 Annexin V, 50 ug/mL	593/614	29011
CF®633 Annexin V, 50 ug/mL	630/650	29008
CF®640R Annexin V, 50 ug/mL	642/662	29014
CF®647 Annexin V, 50 ug/mL	650/665	29003
CF®660R Annexin V, 50 ug/mL	663/682	29069
CF®680R Annexin V, Lyophilized	680/701	29070
CF®680 Annexin V, Lyophilized	681/698	29007
CF®750 Annexin V, Lyophilized	755/777	29006
CF®770 Annexin V, Lyophilized	770/797	29046
CF®790 Annexin V, Lyophilized	784/806	29047
CF®800 Annexin V, Lyophilized	797/816	29078
CF®350 Annexin V, Azide-Free, Lyophilized	347/448	29012R
CF®405M Annexin V, Azide-Free, Lyophilized	408/452	29009R
CF®450 Annexin V, Azide-Free, Lyophilized	405/460	29083R
CF®488 Annexin V, Azide-Free, Lyophilized	490/515	29005R
CF®555 Annexin V, Azide-Free, Lyophilized	555/565	29004R
CF®568 Annexin V, Azide-Free, Lyophilized	562/583	29010R
CF®594 Annexin V, Azide-Free, Lyophilized	593/614	29011R
CF®633 Annexin V, Azide-Free, Lyophilized	630/650	29008R
CF®640R Annexin V, Azide-Free, Lyophilized	642/662	29014R
CF®647 Annexin V, Azide-Free, Lyophilized	650/665	29003R
CF®660R Annexin V, Azide-Free, Lyophilized	663/682	29069R
FITC Annexin V, 50 ug/mL	490/525	29001
Texas Red Annexin V, 50 ug/mL	583/603	29002
R-PE Annexin V	496, 546, 565/578	29045
APC Annexin V	633, 640/660	29057
Biotin Annexin V, 50 ug/mL	N/A	29013
5X Annexin V Binding Buffer	N/A	99902

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Apoptosis & Necrosis Assay Kits

Biotium offers several staining kits that allow concurrent identification of late apoptotic and membrane-compromised necrotic cells by fluorescence microscopy or flow cytometry. These dual staining kits all include green fluorescent CF®488A Annexin V paired with a dead cell-specific nucleic acid dye: either red fluorescent Ethidium Homodimer III (EthD-III), red fluorescent propidium iodide (PI), or far-red fluorescent 7-AAD. EthD-III is a novel membrane-impermeant nucleic acid dye developed at Biotium with higher affinity for DNA and higher fluorescence quantum yield than propidium iodide.

The Apoptotic, Necrotic, and Healthy Cells Quantitation Kit also includes blue fluorescent Hoechst 33342 DNA dye for visualizing all cells (healthy, apoptotic, necrotic/dead) (Fig. 1).

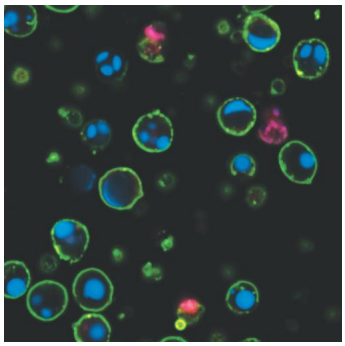


Figure 1. Jurkat cells stained using the Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus after apoptosis induction with staurosporine. Apoptotic cells stain with CF®488A Annexin V (green), necrotic/late apoptotic cells stain with EthD-III (red). All cells are stained with Hoechst (blue).

Apoptosis and Necrosis Combination Kits

Product	Cat. #	Size
Apoptosis & Necrosis Quantitation Kit Plus with CF®488A Annexin V and EthD-III	30065	50 assays
CF®488A Annexin V and 7-AAD Apoptosis Kit	30060	100 assays
CF®488A Annexin V and PI Apoptosis Kit	30061	100 assays
Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus with CF®488A Annexin V, EthD-III and Hoechst	30066	50 assays

Dual Apoptosis Assay Kits

Annexin V conjugated to our deep red CF®594 or far-red CF®640R dyes is offered together with NucView® 488 Caspase-3 Substrate for simultaneous detection of caspase-3 activity and phosphatidylserine exposure by fluorescence microscopy or flow cytometry (see p. 2 for more information on NucView® substrates).

Dual Apoptosis Kits

Product	Cat. #	Size
Dual Apoptosis Assay with NucView® 488 and CF®594 Annexin V	30067	50 assays
Dual Apoptosis Assay with NucView® 488 and CF®640R Annexin V	30073	50 assays

CF® dye TUNEL kits and dUTP conjugates

TUNEL (terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick-end labeling) is highly selective for the detection of apoptotic cells, but not necrotic cells or cells with DNA strand breaks resulting from irradiation or drug treatment. In this assay, TdT enzyme catalyzes the addition of labeled dUTP to the 3' ends of cleaved DNA fragments. Fluorescent dye-conjugated dUTP can be used for direct detection of fragmented DNA by fluorescence microscopy or flow cytometry.

Biotium offers dUTP conjugated to a range of CF® dye colors for fluorescent TUNEL labeling, as well as direct TUNEL kits with green fluorescent CF®488A, red fluorescent CF®594, and far-red fluorescent CF®640R. We also supply dUTP conjugated to classic fluorophores and biotin. Visit www.biotium.com to see our selection of CF® dye conjugated streptavidin, as well as other nucleotide conjugates for probe labeling.

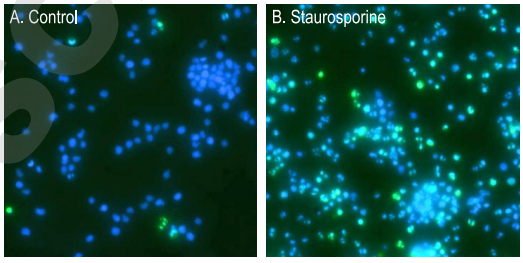


Figure 2. Jurkat cells labeled using the CF®488A TUNEL Assay Apoptosis Detection Kit after A) no treatment or B) apoptosis induction with 1 uM staurosporine for 3 hours. Nuclei are counterstained with DAPI (blue).

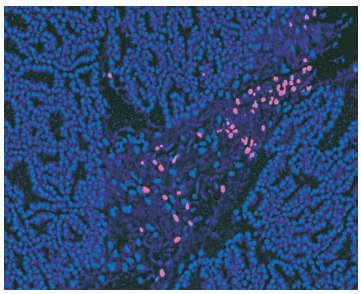


Figure 3. TUNEL staining of paraffin sections of rat mammary gland five days post-weaning (ApopTag® positive control slides, MilliporeSigma) using CF®594-dUTP (red). Nuclei are stained with DAPI (blue).

TUNEL Assays

Product	Ex/Em (nm)	Cat. #	Size
CF®488A TUNEL Assay Kit	490/515	30063	50 reactions
CF®594 TUNEL Assay Kit	593/614	30064	50 reactions
CF®640R TUNEL Assay Kit	642/662	30074	50 reactions

Visit www.biotium.com to see our full selection of biotin- and CF® dye- dUTP conjugates.

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ApopTag is a registered trademark of EMD/Millipore.

Mitochondrial Membrane Potential Dyes

MitoView™ Dyes

MitoView™ dyes are fluorogenic mitochondrial stains for live cells (Fig. 1). The dyes rapidly stain mitochondria without a wash step, and are non-toxic for live cell imaging. They are available with blue, green, far-red, and near-infrared fluorescence. MitoView™ 633 can be used to monitor mitochondrial membrane potenential by microcscopy or flow cytometry (Fig. 2). We also offer MitoView™ Green, a potential-independent mitochondrial dye that can be imaged following mitochondrial depolarization, or after fixation. MitoView™ dyes stain mitochondria in yeast, and also stain bacteria (gram-positive and gram-negative). In addition, we offer a wide selection of classic mitochondrial membrane-potential dyes, including rhodamine 123 and TMRE/TMRM. JC-1 dye can be used for ratiometric measurements of mitochondrial potential, while NAO is a green potential-independent dye that binds cardiolipin phosphoprotein in mitochondria.

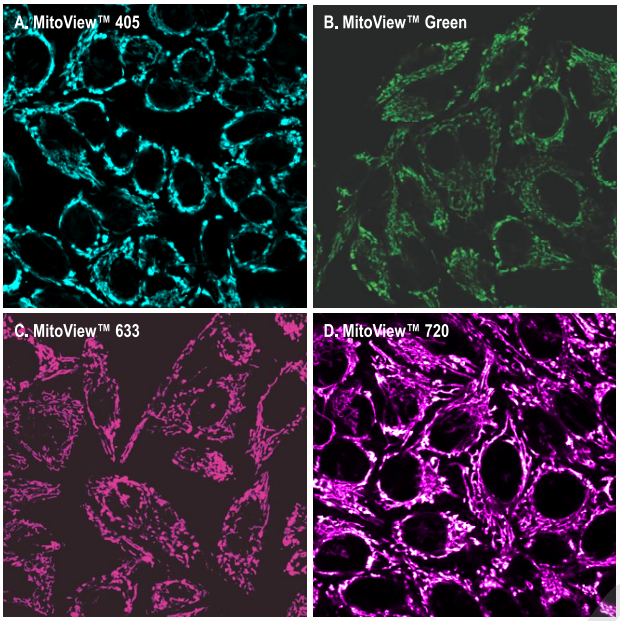


Figure 1. HeLa cells stained with A) MitoView™ 405, B) MitoView™ Green, C) MitoView™ 633, or D) MitoView™ 720.

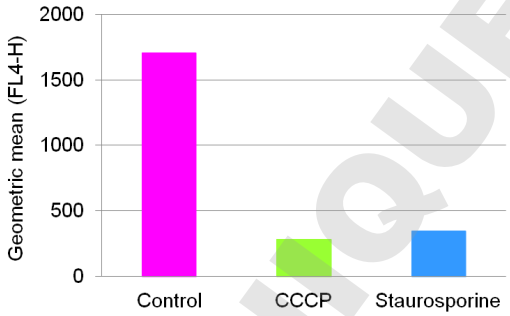


Figure 2. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

MitoView™ Features

- Rapid, no-wash, live-cell stains for mitochondria
- Bright, photostable, and non-toxic
- Available in five colors with blue, green, far-red, or near-IR emission
- Options for mitochondrial potential-dependent or independent staining

MitoView™ Dyes

Product	Ex/Em (nm)	Potential-dependent?	Cat. #
MitoView™ 405	398/440	Partial†	70070
MitoView™ Green	490/523	No	70054
MitoView™ 633	622/648[1]	Yes	70055
MitoView™ 650	644/670	Partial[3]	70075
MitoView™ 720	720/758[2]	Partial[3]	70068

[1] MitoView™ 633 also has visible red fluorescence in the Cy®3/rhodamine channel. It is not recommended for imaging with other visible red probes.
[2] While optimal for Cy®7 settings, MitoView™ 720 is bright enough to be imaged in the Cy®5 channel, and can be combined with visible red fluorescent probes.
[3] Dyes with partial mitochondrial membrane potential dependence localize to the cytoplasm after mitochondrial depolarization, but still retain fluorescence.

More mitochondrial dyes

Product	Ex/Em (nm)	Potential-dependent?	Cat. #
JC-1, chloride salt	510/527; 585/590[4]	Ratiometric[4]	70011
JC-1, iodide salt	510/527; 585/590[4]	Ratiometric[4]	70014
Rhodamine 123	505/534	Yes	70010
TMRE	548/573	Yes	70016
TMRE, 2 mM in DMSO	548/573	Yes	70005
TMRM	548/573	Yes	70017
DASPEI	461/589	Yes	70018
$DiIC_1(5)$	638/658	Yes	70015
Nonyl acridine orange (NAO)	495/522	No	70012

[4] JC-1 forms red fluorescent aggregates in polarized mitochondria, and green fluorescent monomers in cytoplasm

Assay Kits

Product	Features	Cat. #
NucView® 488 and MitoView™ 633 Apoptosis Kit	Two color detection of caspase-3 activity and mitochondrial potential	30062
JC-1 Mitochondrial Membrane Potential Detection Kit	Two-color detection mitochondria polarization/depolarization	30001

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Calcein AM Cell Viability Assay

Calcein AM is a widely used green fluorescent live cell stain. The compound is initially non-fluorescent and membrane permeable compound. Once inside live cells, cytoplasmic esterase activity converts calcein AM to the green fluorescent, membrane-impermeant compound calcein. The dye is only retained in cells with intact plasma membranes, for a true live-cell endpoint assay. The Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells pairs calcein AM with the dead cell dye Ethidium Homodimer III for quantitation of live and dead cells.

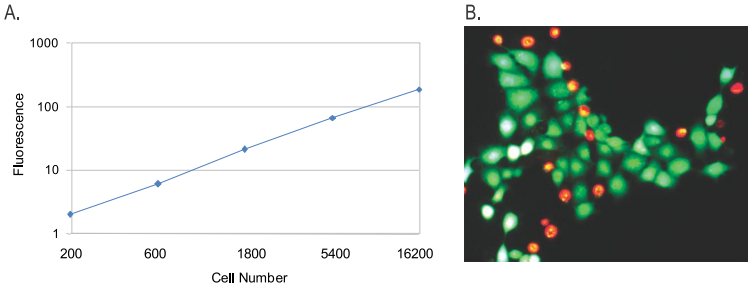


Figure 1. A) Quantitation of HeLa cell numbers in a 96-well assay plate using the Calcein AM Cell Viability Assay Kit. B) Live and dead HeLa cells stained with the Viability/Cytotoxicity Assay for Animal Live & Dead Cells. Live cells are stained green, dead cells are stained red.

ATP-Glo™ Bioluminometric Cell Viability Assay

This assay takes advantage of the ATP-dependent oxidation of D-Luciferin by Firefly luciferase and the resulting production of light in order to assess the amount of ATP in a cell culture, which is proportional to the number of viable cells. The ATP-Glo™ kit can be used to detect as little as a single cell or 0.01 picomole of ATP, with signal linearity for ATP detection within 6 orders of magnitude. This is a flash-type assay designed for detection using a single sample luminometer or a luminometer with an injector in 96-well plate format. The luminescent signal is stable for up to one minute.

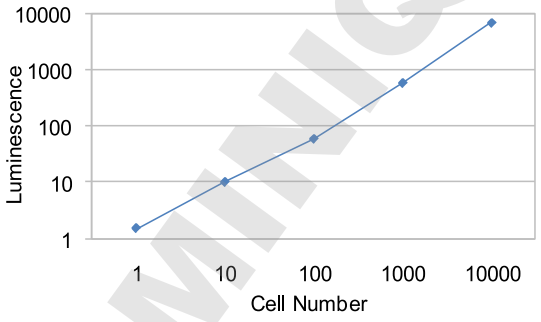


Figure 2. Quantitation of 10-fold serial dilutions of Jurkat cells in suspension using ATP-Glo™ Bioluminetric Cell Viability Assay using a single-tube luminometer.

alamarBlue is a registered trademark of Morphosys UK Ltd.

Resazurin, MTT, and XTT Viability Assays

MTT, XTT, and resazurin (alamarBlue®) are reduced by mitochondrial metabolic activity to yield colored or fluorescent products, and thus are useful for assaying cell viability and quantitating cell number. MTT and XTT reduction products are measured by absorbance; MTT requires cell lysis before absorbance measurement, while XTT does not. Resazurin is a non-fluorescent blue dye that is reduced to the pink fluorescent compound resorufin, which can be measured by fluorescence or absorbance in intact cells.

ViaFluor® SE Cell Proliferation Kits

ViaFluor® SE Cell Proliferation Dyes diffuse passively into cells and covalently label intracellular proteins throughout the cell. They can be used as cell-filling stains for imaging morphology, or to track cell division by dye dilution. With each cell division, daughter cells inherit roughly half of the fluorescent label, allowing the number of cell divisions to be detected by the appearance of successively dimmer fluorescent peaks on a flow cytometry histogram (Fig. 3). The stains also can be used for long-term cell tracking by microcopy, and for stable labeling of cells in co-culture. Because the staining is covalent, it has excellent tolerance for fixation and permeabilization.

ViaFluor® CFSE is the classic cell proliferation dye, detected in the FITC channel. Biotium created ViaFluor® 488, a new improved green dye that is less toxic, less leaky and more fixable than CFSE. We also offer blue fluorescent ViaFluor® 405 for the violet laser. ViaFluor® 405 has improved brightness and less toxicity than CFSE.

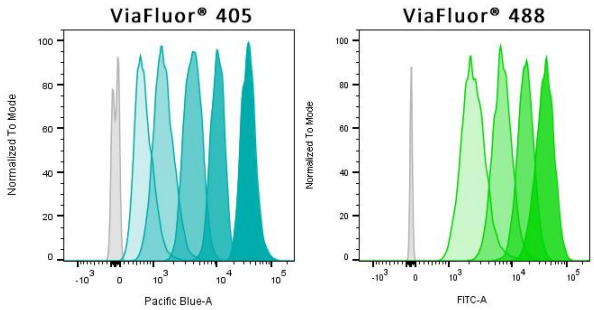


Figure 3. Cell division tracking in Jurkat cells over successive days. Cells were labeled with ViaFluor® 405 (left) or ViaFluor® 488 (right) on day 0, and analyzed by flow cytometry on each following day. Each successively dimmer peak represents one cell division. Unstained cells are in gray.

Cellular Viability Assays

Product	Cat. #
Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	30002
Calcein AM Cell Viability Assay Kit	30026
PathoGreen™ Histofluorescent Stain, 1000X in water	80027
Resazurin Cell Viability Assay Kit	30025
MTT Cell Viability Assay Kit	30006
XTT Cell Viability Assay Kit	30007
ATP-Glo™ Bioluminometric Cell Viability Assay Kit	30020
ViaFluor® 405 SE Cell Proliferation Kit	30068
ViaFluor® 488 SE Cell Proliferation Kit	30086
ViaFluor® CFSE Cell Proliferation Kit	30050

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Dead Cell Nucleic Acid Stains

Membrane-impermeant nucleic acid dyes have very low fluorescence until they bind DNA or RNA. They are used for selective staining of necrotic and late apoptotic cells that have leaky plasma membranes, and can be used in homogenous assays or real-time cell imaging without a wash step. The cyanine-based nucleic acid stains have high affinity and excellent brightness. Biotium offers a wide variety of dead cell nucleic acid stains in several colors, as well as Apoptosis & Necrosis Staining Kits with dead cell stains and apoptosis markers (see page 5) and Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells with calcein AM for live cell staining and Ethidium Homodimer III for dead cell staining (see page 7).

Dead Cell Nucleic Acid Stains

Product	Ex/Em (nm)	Features	Cat. #
Oxazole Blue, 1mM in DMSO	Blue 435/455	• Blue cell impermeant dye • Equivalent to PO-PRO™-1 Iodide	40091
NucSpot® 470, 1000X in DMSO	Green 460/546	• Green cell impermeant dye • Nuclear-specific counterstain in fixed cells • Excellent match for blue LED excitation sources	40083
Oxazole Yellow, 1mM in DMSO	Green 491/509	• Green cell impermeant dye • Selectively stains early apoptotic cells • Equivalent to YO-PRO®-1 Iodide	40089
TO Iodide, 1mM in DMSO	Green 515/533	• Green cell impermeant dye • Equivalent to TO-PRO®-1 Iodide	40088
Thiazole Orange Homodimer, 1 mM in DMSO	Green 514/533	• High affinity dimeric cyanine dye • Dead cell stain and electrophoresis dye • Equivalent to TOTO®-1 Iodide	40079
Propidium Iodide, 100 mg	Red 535/617	• Widely used dead cell stain • Can be excited by 488 nm laser line for detection in the PE channel by flow cytometry • Useful for cell cycle analysis in fixed cells (with RNase treatment)	40016
Propidium Iodide 1 mg/mL in Water			40017
Propidium Iodide 50 ug/mL in Buffer			40048
Ethidium Homodimer I, 1 mg	Red 528/617	• High-affinity membrane-impermeant nucleic acid stain • >30-fold fluorescence enhancement upon binding to DNA/RNA • High-purity grade not available from other manufacturers	40010
Ethidium Homodimer I, 2 mM in DMSO			40014
Ethidium Homodimer III, 1 mg	Red 530/620	• Developed at Biotium as an alternative to Ethidium Homodimer I • 45% brighter than EthDI when bound to DNA	40050
Ethidium Homodimer III, 1 mM in DMSO			40051
7-AAD, 1 mg	Far-red 546/667	• Far-red dye for flow cytometry detection in the PE-Cy®5 channel • Can be excited by the 488 nm or 532 nm laser line • Useful for cell cycle analysis in fixed cells	40037
7-AAD, 1 mg/mL Solution			40084
NucSpot® Far-Red, 1000X in DMSO	Far-red 597/667	• Designed as improved replacement for 7-AAD • For flow cytometry in the PE-Cy®5 or APC channel • Less bleed into the PE-Texas Red® channel	40085
RedDot™2 Far-Red Nuclear Stain	Far-red 665/695	• Far-red cell impermeant dye for the Cy®5 channel • Nuclear-specific counterstain in fixed cells • Replaces Draq7™	40061
Thiazole Red, 1 mM in DMSO	Far-red 642/661	• Far-red cell impermeant dye for the Cy®5 channel • Dead cell stain and electrophoresis dye • Equivalent to TO-PRO®-3 Iodide	40087
Thiazole Red Homodimer, 1 mM in DMSO	Far-red 642/660	• High affinity dimeric cyanine dye for the Cy®5 channel • Useful dead cell stain • Equivalent to TOTO®-3 Iodide	40080

PathoGreen™ Histofluorescent Stain

PathoGreen™ Histofluorescent Stain is an anionic green fluorescent dye functionally similar to Fluoro-Jade® dyes. These dyes stain degenerating neurons after exposure to a variety of neurotoxic insults. Staining can be done in live or fixed cells or tissue sections (Fig. 1). The mechanism of neuronal staining by anionic fluorescent dyes has not been determined. It has been proposed that the negatively charged dyes bind to positively charged polyamines or other molecules specifically generated in dying neurons.

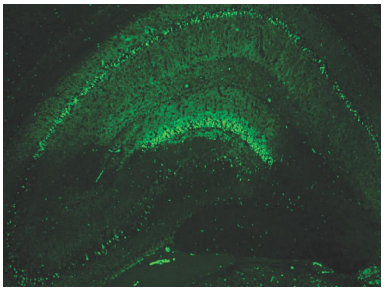


Figure 1. A section of mouse hippocampus stained with PathoGreen™ Histofluorescent Stain. Degenerating neurons are stained green.

Fluoro-Jade is a registered trademark of Histo-Chem, Inc. SYBR, PO-Pro, Texas Red, TOTO, TO-PRO, and YO-PRO are trademarks or registered trademarks of Thermo Fisher Scientific. Cy dye is a registered trademark of GE Healthcare. Draq7 is a trademark of Biostatus Ltd.

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Live-or-Dye™ Fixable Dead Cell Stains

Live-or-Dye™

Live-or-Dye™ Fixable Viability Staining Kits are designed for discrimination between live and dead cells by flow cytometry and microscopy. Dead cell stains are useful probes to include when analyzing cell surface protein expression by flow cytometry (Fig. 1), because they allow intracellular fluorescence signal from dead cells with permeable plasma membranes to be excluded from analysis. Live-or-Dye™ Fixable Viability Stains are cell membrane-impermeant; they enter dead cells that have compromised membrane integrity and covalently label free amines on intracellular proteins. Live-or-Dye™ Fixable Viability Staining Kits can also be used to discriminate live from dead cells by microscopy (Fig. 2A). Live-or-Dye™ staining is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

Live-or-Dye NucFix™ Red

Live-or-Dye NucFix™ Red is a unique, cell membrane-impermeant dye that specifically stains the nuclei of dead cells (Fig. 2B). The dye is able to enter into dead cells that have compromised membrane integrity and covalently label the cell nucleus. Unlike other commonly used nuclear stains such as propidium iodide or DRAQ®7, NucFix™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

Live-or-Dye™ Fixable Stains for Dead Cells

- **Variety:** 10 bright colors across the spectrum
- **Fixable:** Live/dead signal unaffected by fixation
- **Versatile:** For flow cytometry and microscopy
- **Affordable**: Lower cost than Thermo Fisher Scientific LIVE/DEAD® stains
- **Spectral Flow:** Compatible dyes available

Live-or-Dye Nucfix™ Red

- A unique cell membrane-impermeant dye that stains nuclei of dead cells
- Fixable, unlike other commonly used nuclear stains such as propidium iodide or DRAQ7™

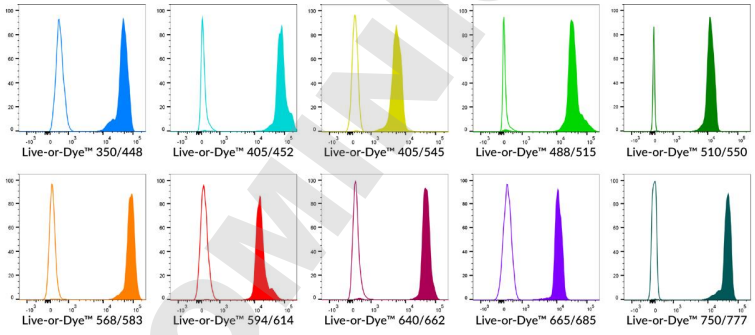
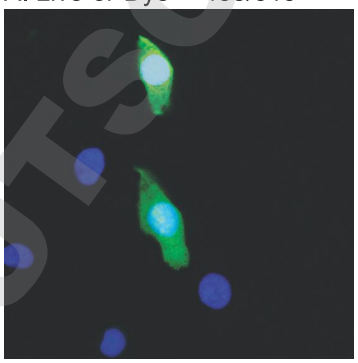


Figure 1. Discrimination of live and dead Jurkat cells by flow cytometry using Live-or-Dye™ Fixable Viability Stains. Heat killed cells (solid peaks) showed much higher fluorescence intensity compared to live cells (white peaks).

A. Live-or-Dye™ 488/515



B. Live-or-Dye NucFix™ Red

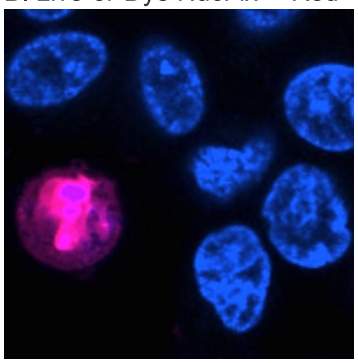


Figure 2. HeLa cells were treated with ethanol to kill a fraction of the cells. The cells were stained with A) Live-or-Dye™ 488/515 or B) Live-or-Dye NucFix™ Red. Nuclei were counterstained with Hoechst.

Live-or-Dye™ Kits

Kit Name	Ex/Em (nm)	Cat. # 200 Rxns	Cat. # 50 Rxns	Apps
Live-or-Dye™ 350/448	347/448 nm	32002	32002-T	FC
Live-or-Dye™ 405/452	408/452 nm	32003	32003-T	FC
Live-or-Dye™ 405/545	395/545 nm	32009	32009-T	FC
Live-or-Dye™ 488/515	490/515 nm	32004	32004-T	FC, M
Live-or-Dye™ 510/550	516/549 nm	32012	32012-T	SC
Live-or-Dye™ 568/583	562/583 nm	32005	32005-T	FC, M
Live-or-Dye™ 594/614	593/614 nm	32006	32006-T	FC, M
Live-or-Dye™ 640/662	642/662 nm	32007	32007-T	FC, M
Live-or-Dye™ 665/685	667/685 nm	32013	32103-T	SC
Live-or-Dye™ 750/777	755/777 nm	32008	32008-T	FC
Live-or-Dye NucFix™ Red	520/610 nm	32010	32010-T	FC, M

Apps= Validated Applications; FC= flow cytometry; SC= spectral cytometry; M= microscopy

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PMAxx™ and PMA Dyes for Viability PCR

Viability PCR (v-PCR)

Viability PCR is a powerful technology for the sensitive and rapid detection of viable microorganisms. Unlike time-consuming culturing procedures, qPCR is a fast and sensitive method of detection. However, normal qPCR does not distinguish between live and dead cells. With v-PCR using PMAxx™ or PMA, you get the speed, sensitivity and specificity of PCR, plus quantifiable viability. And because no culturing is required, you can detect viable but not culturable (VBNC) bacteria.

How does v-PCR work?

PMAxx™ and PMA are photoreactive dyes with high affinity for DNA. The dyes intercalate into dsDNA and form a covalent linkage upon exposure to intense visible light. PMAxx™ and PMA inhibit PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases. Because PMAxx™ and PMA are cell membrane-impermeant, when a sample containing both live and dead bacteria is treated with dye, only dead bacteria with compromised cell membranes are susceptible to DNA modification (Fig. 1). In a real-time PCR reaction, dead cell DNA will show delayed amplification and higher Ct than live cells. In a mixed population, v-PCR permits quantitation of cell viability. The v-PCR technology can be applied not only to bacteria but to other cell types as well.

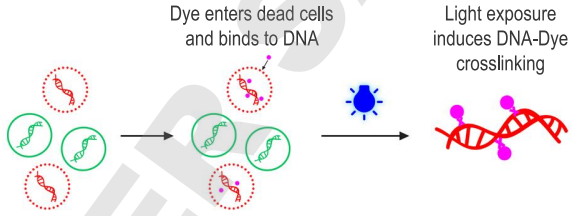


Figure 1. Mechanism of PMA and PMAxx™. The cell membrane-impermeant dyes PMA and PMAxx™ selectively and covalently modify DNA from dead bacteria with compromised membranes while leaving DNA from viable cells intact.

PMAxx™ vs PMA

Since Biotium developed PMA in 2006, it has been used extensively in many applications and in hundreds of publications. However there are cell types and conditions in which dead cell DNA inactivation by PMA is incomplete, which could lead to false positive results. After extensive testing, scientists at Biotium have invented a new dye called PMAxx™ that has the same spectral properties and is even more effective than PMA at live/dead discrimination by viability PCR (Fig. 2).

v-PCR LED Photolysis Devices



PMA-Lite™:

- Holds up to 18 microcentrifuge tubes
- Bright, long-lasting blue LED lights
- Fan ensures temperatures lower than 37°C



Glo-Plate™ Blue:

- Flat illumination surface fits microplates
- Bright, long-lasting blue LED lights
- Surface stays cool during light exposure

Strain-specific v-PCR kits available for:

- *Salmonella enterica*
- *Escherichia coli*
- *Escherichia coli* O157:H7
- *Listeria monocytogenes*
- *Legionella pneumophila*
- *Mycobacterium tuberculosis*
- *Staphylococcus aureus*
- Methicilin-resistant *Staphylococcus aureus* (MRSA)

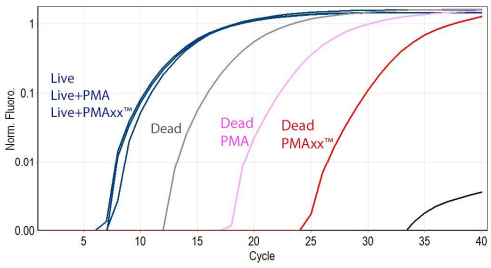


Figure 2. Live or heat-killed *Bacillus subtilis* were treated with PMA or PMAxx™, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 500-bp fragment of *B. subtilis* DNA. qPCR of dead cells treated with PMAxx™ showed a significant further delay (>7 Ct) compared to dead cells treated with PMA.

Ordering Information

Product Name	Cat. #	Unit Size
PMAxx™ Dye, 20 mM in dH_2O	40069	100 uL
PMA Dye	40013	1 mg
PMA Dye, 20 mM in dH_2O	40019	100 uL
PMA-Lite™ LED Photolysis Device	E90002	1 device
Glo-Plate™ Blue	E90004	1 device
PMA Enhancer for Gram-Negative Bacteria	31038	16 mL
Real-Time Bacterial Viability Kit - Salmonella (InvA)	31033	200 assays
Real-Time Bacterial Viability Kit - M. tuberculosis (groEL2)	31034	200 assays
Real-Time Bacterial Viability Kit - Staph. aureus (nuc)	31035	200 assays
Real-Time Bacterial Viability Kit - MRSA (mecA)	31036	200 assays
Real-Time Bacterial Viability Kit - E. coli (uidA)	31050	200 assays
Real-Time Bacterial Viability Kit - E. coli O157:H7 (Z3276)	31037	200 assays
Real-Time Bacterial Viability Kit - Listeria monocytogenes (hly)	31051	200 assays
Real-Time Bacterial Viability Kit - Legionella pneumophila (mip)	31053	200 assays
Viability PCR Starter Kits - Any Cells	31075	200 assays
Viability PCR Starter Kits - Gram Negative Bacteria	31076	200 assays

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Bacteria Viability Dyes and Kits

Live-or-Dye™ Fixable Viability Staining Kits utilize dead-cell-specific fixable dyes (Fig. 1). They are useful for flow cytometry and microscopy and available in 10 bright, photostable colors. See p. 9 for more information on Live-or-Dye™ stains. CTC is a fluorescent dye that has been used to evaluate the respiratory activity in bacteria. Healthy cells will reduce CTC into an insoluble red product. The Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells features dual staining: DMAO for live cells, and EthD-III for dead cells (Fig. 2).

Combination Gram Staining and Viability Kits

It can be useful to distinguish live bacteria from dead, as well as gram positive from gram negative, in the same sample. Our combination bacterial viability and fluorescent Gram staining kits can help (Fig. 3). Our fluorescent Gram stains utilize fluorescently-labeled wheat germ agglutinin (WGA) to selectively stain the cell walls of gram-positive bacteria.

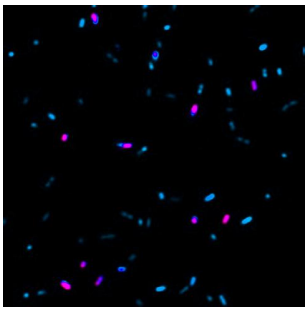


Figure 1. Live and heat-killed *E. coli* stained with Live-or-Dye™ 568/583 (red) and DAPI (blue).

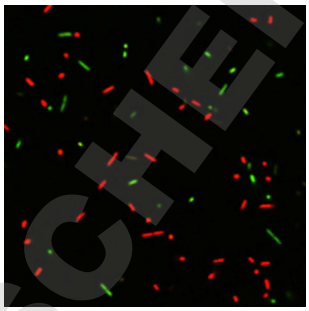


Figure 2. Live and heat-killed *E. coli* stained with DMAO, marking live cells (green) and EthD-III, marking dead cells (red).

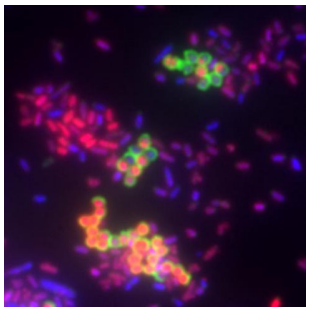


Figure 3. Bacterial Viability and Gram Stain Kit. CF®488A-WGA, EthD-III and DAPI.

Bacteria Viability Stains

Product	Features	Cat. #
CTC (5-Cyano-2,3-ditolyl tetrazolium chloride)	Forms insoluble red product in respiring cells	10063
Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells	DMAO to stain all cells and EthD-III for dead cells	30027
Bacterial Viability and Gram Stain Kit	WGA for gram stain, EthD-III for dead cells, and DAPI for all cells	32001

Yeast Viability Dyes and Kits

It is often useful to distinguish live yeast cells from dead, or identify cells that are metabolically active. Our selection of yeast viability dyes and kits can help.

Yeast Viability Products

Live-or-Dye™ Fixable Viability Staining Kits:

- Fixable and dead-cell-specific
- Suitable for flow cytometry and microscopy
- Available in 10 colors as well as NucFix™ Red

ViaVac™ Red/Green, a vacuolar cell vitality dye:

- Passive diffusion leads to green cytoplasmic staining
- Red vacuole staining in metabolically active yeast

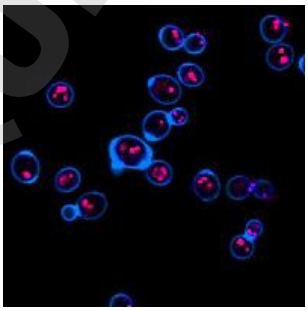


Figure 4. Yeast Vitality Staining Kit, ViaVac™ Red/Green (red, healthy vacuolar tubules) and Calcofluor White (blue, cell wall).

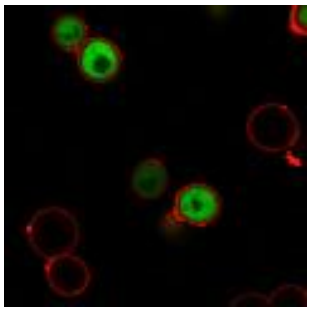


Figure 5. Yeast Viability Staining Kit, CF®-ConA (red, cell wall) and Live-or-Dye™ (green, dead cell cytoplasm).

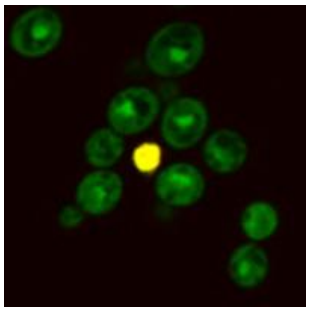


Figure 6. Yeast Fixable Live/Dead Staining Kit, Thiazole Orange (green, all cells) and Live-or-Dye™ 568/583 (red, dead cell cytoplasm). Overlapping signal appears yellow.

Yeast Viability Stains

Product	Description	Cat. #
ViaVac™ Red/Green, 10 mM in DMSO	Yeast vital dye	29068
Yeast Vitality Staining Kit	ViaVac™ Red/Green and Calcofluor White	31062
Yeast Viability Staining Kit	CF® ConA and Live-or-Dye™ combinations	31063
Yeast Fixable Live/Dead Staining Kit	Thiazole Orange and Live-or-Dye™ 568/583	31064

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