Our Mission

AAT Bioquestfi is committed to constantly meet or exceed its customer s requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquestfi, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), uorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest o ers a rapidly expanding list of enabling products. Besides the standard catalog products, we also o er custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

Table of Contents

CUSTOMER SERVICE & ORDERING INFORMATION

AAT Bioquest Corporate Headquarter:
520 Mercury Drive
Sunnyvale, CA 94085, USA
Phone: 800-990-8053 (US and Canada)
408-733-1055 (International)
Fax: 408-733-1304
Website: www.aatbio.com
E-mails: info@aatbio.com (inquire)
sales@aatbio.com (quote request)
support@aatbio.com (technical support)

International Distributors:
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Cell Explorer™
Cell Meter™
CytoTell™
CytoTrace™
CytoCalcein™
Live or Dead™
Nuclear Blue™
Nuclear Green™
Nuclear Orange™
Nuclear Red™
PhosphoWorks™

TERMS AND CONDITIONS OF SALE

1. Prices, Orders and Changes: Prices shown are in US currency. Please call us for current prices if you require this information prior to placing your order. We guarantee our written quotations for 60 days. You may not cancel purchase orders unless such cancellation is expressly agreed by us. In such event, you will be advised of the total charge for such cancellation. You agree to pay such charges, including, but not limited to, storage and shipment costs, costs of producing non-standard materials, costs of purchasing non-returnable materials, cancellation costs imposed on us by our suppliers, and any other cost resulting from cancellation of this order.

2. Delivery: In most cases, we use standard overnight or two-day Federal Express delivery (or equivalent). All shipping charges billed are the responsibility of the customer and are normally prepaid by AAT Bioquest, Inc. and added to the invoice. We reserve the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Partial shipments of available items are made when another item is backordered. Please inspect your packages upon receipt. If the goods have been damaged in transit, we can assist you in filing a claim with the carrier. You shall notify us in writing of any claims for shortages, defects or damages and shall hold the goods for our written instructions concerning disposition. Any claims for such errors must be made within 10 business days. If it is our error, we will do whatever is necessary to ship the correct products as soon as possible. If you shall fail to notify us any defects within 10 days after the goods have been received, such goods shall conclusively be deemed to conform to the terms and conditions and to have been irrevocably accepted by the buyer.

3. Payment: Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

4. Warranties: The products shipped by AAT Bioquest are warranted to conform to the chemical or biological descriptions provided in our publications. This warranty is exclusive, and we makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose. Our sole and exclusive liability and your exclusive remedy with respect to products proved to our satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in our sole discretion, upon the return of such products in accordance with our instructions. We will not be liable for any incidental, consequential or contingent damages involving their use.

5. Returns: We must authorize any returns. We will not accept return shipments unless we have given prior written permission and shipping instructions. Goods may not be returned for credit except with our permission, and then only in strict compliance with our return shipment instructions. Any returned items may be subject to a 20% restocking fee. In many cases, items ordered in error cannot be returned because of the sensitive nature of many of our products and the difficulty and expense of requalifying returned items. If items are accepted for return, they must be in new, unopened, unused and undamaged condition, and you will be charged a per-unit 20% restocking charge.

6. Use of Our Products: Our products are used ONLY for laboratory research and development purposes. We realize that, since our products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. You assume responsibility to assure that the products purchased from us are approved for use under TSCA, if applicable. You have the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from us. You also have the duty to warn your customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

7. Patent Disclaimer: We do not warrant that the use or sale of our products will not infringe the claims of any United States or other patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

8. Miscellaneous: We reserve the right to discontinue our products or change specifications or prices of our products and to correct any errors or omissions at any time without incurring obligations.

Cell Viability and Cytotoxicity Assay Reagents

Introduction

Cells perform many important functions, such as secretion, signal transduction and cell proliferation, which are essential for their growth, maintenance and survival. These processes are often coupled to changes in intracellular radicals, transmembrane potentials and free-ion concentrations. Employing the correct stimulant can trigger similar changes, and with the appropriate responsive fluorescent indicator these changes can be monitored to assess cell viability, cytotoxicity and apoptosis. This approach is frequently utilized in cancer biology, drug-discovery and various other cell-based studies.

Although cell viability, cytotoxicity and cell proliferation assays are commonly grouped together, each are designed to monitor and measure different parameters. For instance:

- Cell viability and cytotoxicity assays measure the rate of ongoing cellular activities e.g., cellular metabolism or enzyme activity and test for cytolysis or membrane leakage.
- Cell proliferation and cell cycle assays monitor the growth of a cell population or detect the generation of daughter cells.

Since no single assay can comprehensively assess the health of a cell population, it is most effective to use a combination of several different methods (including apoptosis assays) in your experiment. AAT Bioquest offers a broad range of reagents and kits for assessing cell viability, cytotoxicity and cell proliferation. The following sections discuss the various parameters used to measure cell health, and which products are best-suited for their respective assessment.

Cell Viability/Cytotoxicity Assay Reagents

Cell viability and cytotoxicity assays are frequently employed together to assess the health of a cell population at various stages in cell-based or drug discovery studies. Cell viability assays are used to evaluate the percentage of living cells in a sample population by monitoring characteristics associated with healthy cell function e.g., cell metabolism and enzyme activity. Determining a cell population's viability is crucial prior to beginning any cell-based study because healthy cells are necessary to produce accurate and reliable results. Cytotoxicity assays are designed to quantify the proportion of live and dead cells in a population by assessing damage to cellular membranes. In drug discovery, both cell viability and cytotoxicity assays aid in evaluating the effectiveness of novel pharmacological compounds.

Our selection of fluorimetric-based assay reagents and kits for assessing cell viability and cytotoxicity are reliable and easy to perform. They are more affordable and less hazardous than radioisotopic techniques and more sensitive than colorimetric assays. This section focuses on highlighting our vast collection of stand-alone reagents for assessing viability and cytotoxicity suitable for a variety of cell types and detection formats. Following this discussion of individual reagents, the next section will describe each of our viability and cytotoxicity kits. For a brief overview of these methods and their respective reagents see **Table 1**.

Table 1. Common parameters and methods for assessing cell viability and cytotoxicity.

Assay	Parameter	Method	Example of appropriate Reagent/Kit
Cell Viability	Analyzes ongoing cellular metabolism and enzyme activity to quantify cell viability	Dyes responsive to cellular enzyme activity	ReadiUse™ WST-8 (Cat# 15705) Cell Meter™ Colorimetric WST-8 Kit (Cat# 22770)
		Detect mitochondrial membrane potential gradients using potentiometric dyes	JC-1 (Cat# 22200) JC-10™ (Cat# 22204)
		Cellular esterases cleaved fluorescent dyes	Calcein, AM (Cat# 22002)
		ATP and ADP assays	D-Luciferin (Cat# 12507) Luciferase (Cat# 12500)
		Measure oxidation or reduction	Resazurin (Cat# 15700)
Cell Cytotoxicity	Analyzes damage to cell membranes to determine the number of live and dead cells in a population	Measure the activity of intracellular enzymes	Cell Meter™ Cell Cytotoxicity Assay Kit (Cat# 22781)
		Assess membrane damage using cell-impermeable nucleic acid stains	Nuclear Blue™ DCS1 (Cat# 17548)
		Amine-reactive dyes to discriminate live from dead cells in a population by fluorescence intensity	Live or Dead™ Fixable Dead Cell Staining Kit (Cat# 22600)

Fluorogenic Esterase Substrates for Cell Viability/ Cytotoxicity Assays

A variety of fluorogenic esterase substrates (**Table 2**) including Calcein AM and various fluorescein diacetate derivatives serve as viability probes for assessing enzymatic activity and cell-membrane integrity. The properties of these probes facilitate their passive diffusion across intact plasma membranes of most cell types. Once inside the cell, these non-fluorescent substrates are hydrolyzed by intracellular esterases into fluorescent products that are retained by cells with intact plasma membranes. On the contrary, cells that are dead or have damaged membranes will rapidly leak both the unhydrolyzed substrates and their products from their cytosol. Fluorogenic esterase substrates are detectable with fluorescence microscopes, flow cytometers, and fluorescence microplate readers.

Calcein AM: Esterase Substrates

Calcein AM is a hydrophobic compound commonly used for measuring cell viability in live cells. Conjugation of AM esters to Calcein facilitates permeation of healthy cell membranes, and delays fluorescent signal generation until the dyes are activated intracellularly by esterase-catalyzed hydrolysis. The esterase activity is proportional to the number of viable cells, and thus directly related to the fluorescence intensity of the Calcein product. Calcein exhibits a maximum excitation and emission of 495 nm and 515 nm, respectively. Since Calcein shares similar spectral properties with GFP and FITC it is nearly impossible to use in multicolor analysis.

To address this limitation, AAT Bioquest has developed a comprehensive set of Calcein analogs available in a variety of

Table 2. Esterase substrates for cell viability and cytotoxicity assays.

Esterase Substrate	Properties in Cell	Ex (nm)	Em (nm)
BCECF, AM (Cat#21202)	• pH-sensitive fluorescent indicator • cell-permeable • dye released intracellularly	503	528
Calcein, AM (Cat#22002)	• cell-permeable • low cytotoxicity compared to BCECF and CFDA • dye released intracellulary	495	515
CFDA [5-(and 6)-Carboxyfluorescein diacetate] (Cat#22021)	• cell-permeable • amine-reactive FDA derivative • pH-sensitive	494	521
CytoTrace™ Green CMFDA (Cat#22017)	• cell-permeable • visible in daughter cells after multiple generations • long term tracer - fluorescent and viable for 24 hrs • fixable with formaldehyde or glutaraldehyde	494	521
CytoTrace™ Orange CMTMR (Cat#22014)	• cell-permeable, rhodamine analogs • signal visible in daughter cells after multiple generations • long term tracer - fluorescent and viable for 72 hrs • stable, non toxic and brightly fluorescent at physiological pH	541	565
CytoTrace™ Red CFDA (Cat#22016)	• cell permeable, longer wavelength FDA analog • signal visible in daughter cells after multiple generations • long term tracer - fluorescent and viable for 24 hrs • fixable with formaldehyde or glutaraldehyde • adaptable for multiplexing applications	560	574
CytoTrace™ Red CMTPX (Cat#22015)	• cell-permeable, rhodamine analogs • signal visible in daughter cells after multiple generations • long term tracer - fluorescent and viable for 72 hrs • stable, non toxic and brightly fluorescent at physiological pH	577	602
Fluorescein Diacetate (FDA) (Cat#22020)	• cell permeable hydrophobic fluorescein derivative • poorly retained in cells • pH sensitive fluorescence	494	521

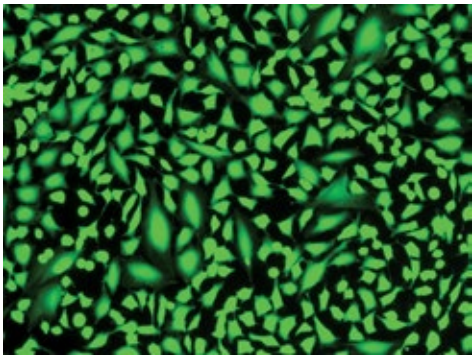


Figure 1.1 Fluorescence image of HeLa cells stained with Calcein UltraGreen™ AM (Cat# 21905) in a Costar black wall/clear bottom 96-well plate. After washing cells in cell viability indicator-free buffer, cells were monitored using a fluorescence microscope with FITC filter set.

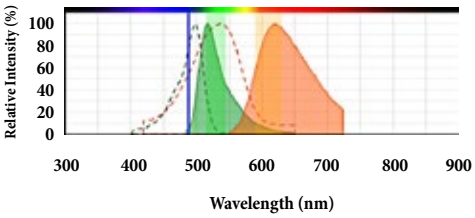


Figure 1.2 Excitation and emission spectrum of fluorescein diacetate (Cat# 22210, Green) and propidium iodide (Cat# 17517, Red) illustrating the clear spectral separation that enables two-color assessment of live and dead cells in a population. Both fluorescein diacetate and propidium iodide are efficiently excited by the 488 nm argon-ion laser. Fluorescein diacetate can be visualized with a FITC filter set. Propidium iodide can be visualized with a Texas Red filter set.

fluorescence emissions **Figure1.3**. For example, using Calcein UltraBlue™ AM (Ex/Em = 360/445 nm) in combination with a green fluorescence indicator such as cell-impermeant Nuclear Green™ DCS1 (Ex/Em = 503/526 nm) enables multicolor viability and cytotoxicity assessment.

CytoCalcein™ Violet 450 and CytoCalcein™ Violet 500 probes have been optimized for efficient excitation with the 405 nm violet diode laser commonly equipped in flow cytometers. Upon hydrolysis and excitation, CytoCalcein™ Violet 450 generates a blue fluorescence, whereas CytoCalcein™ Violet 500 fluoresces green.

Fluorescein Diacetate

Fluorescein Diacetate (FDA) is a cell-permeant and non-fluorescent fluorescein derivative that can be used to assess cell viability. Once inside the cell, esterases hydrolyze the diacetate groups to produce fluorescein. Upon excitation by the 488 nm argon laser, FDA will fluoresce green. FDA may be used in combination with propidium iodide (PI) to determine cell viability. This two-color separation of non-viable and viable cells may provide a more accurate quantitation of cell viability versus single color analysis. Because fluorescein suffers from high rates of photobleaching and poor cell retention, it is recommended to use carboxyfluorescein diacetate or CytoTrace™ dyes as an alternative.

Carboxyfluorescein Diacetate

Carboxyfluorescein Diacetate (CFDA) is spectrally identical to FDA and can be used a fluorescent cell viability indicator. Hydrolysis of CFDA by intracellular esterases produces carboxyfluorescein, which when excited by the 488 nm argon laser, fluoresces green. Compared to fluorescein, carboxyfluorescein contains extra negative charges and can be better retained in cells.

CytoTrace™ Dyes

Our CytoTrace™ dyes are a series of cell-permeant thiol-reactive fluorescent indicators for assessing cell viability. In addition they can serve as long-term cell proliferation tracers. CytoTrace™ dyes are well-retained in many live cells through several generations without being transferred to adjacent cells in a population. They can be

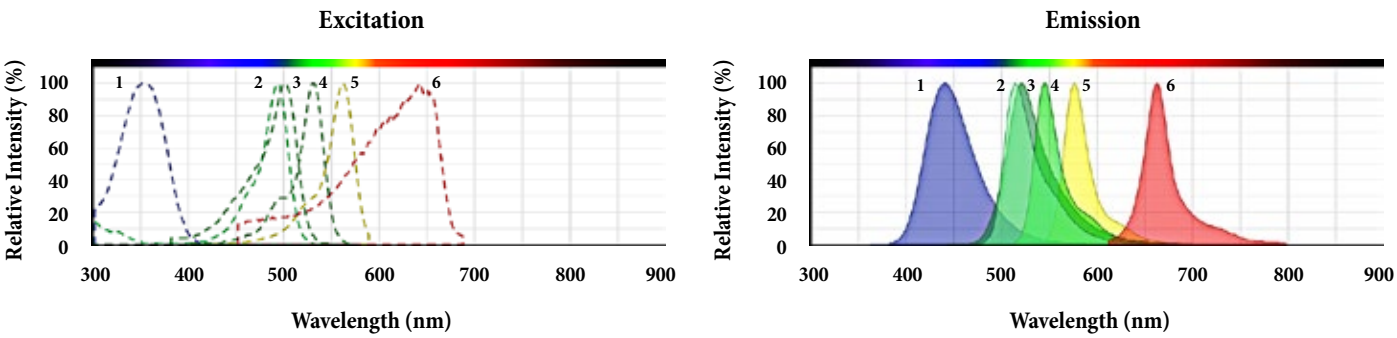


Figure 1.3 Excitation (left) and emission (right) spectrum of Calcein AM dyes. **1)** Calcein Blue AM (Cat#22006) **2)** Calcein UltraGreen™ AM (Cat#21905) **3)** Calcein AM (Cat#22002) **4)** Calcein Orange™ diacetate (Cat#22009) **5)** Calcein Red™ AM (Cat#21900) **6)** Calcein Deep Red™ AM (Cat#21902)

monitored using fluorescence microscopy or flow cytometry.

CytoTrace™ Green CMFDA and CytoTrace™ Red CFDA freely diffuses across cell membranes, where upon reaction with cellular components they are converted into cell-impermeant products. Cells that are loaded with these indicators are typically fluorescent and viable for at least 24 hours. The staining pattern can be fixed with formaldehyde or glutaraldehyde for signal amplification and other downstream applications.

CytoTrace™ Orange CMTMR and CytoTrace™ Red CMTPX are cell-permeant, highly fluorescent rhodamine dyes that do not require esterase cleavage to activate their fluorescence. Cells that are loaded with these indicators are typically fluorescent and viable for at least 72 hours. These dyes are stable, nontoxic at working concentrations and brightly fluorescent at physiological pH. The excitation and emission spectra of CytoTrace™ CMTMR and CMTPX dyes are well separated from GFP allowing for multiplexing analysis.

By combining CytoTrace™ dyes with membrane-impermeant nucleic acid stains such as PI or our Nuclear DCS1 dyes should permit for a relatively long term cell viability and cytotoxicity quantification.

Table 3. CytoTrace™ Dyes for assessing cell viability and long-term cell proliferation.

Catalog #	CytoTrace™ Dye	Ex/Em (nm)
22017	CytoTrace™ Green CMFDA	403/445
22014	CytoTrace™ Orange CMTMR	528/541
22015	CytoTrace™ Red CMTPX	575/600
22016	CytoTrace™ Red CFDA	637/650

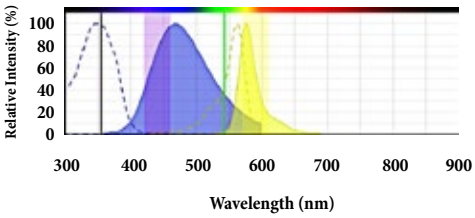


Figure 1.4 Excitation and emission spectrum of Nuclear Blue™ DCS1 (Cat# 17548, Blue) and CytoTrace™ Red CFDA (Cat# 22016, Yellow) illustrating the clear spectral separation that enables two-color assessment of live and dead cells in a population. Nuclear Blue™ DCS1 can be efficiently excited by the common 350 nm UV excitation and visualized with a DAPI filter set. CytoTrace™ Red CFDA can be efficiently excited by the 543 nm helium-neon laser and visualized with a Cy3/TRITC filter set.

Nucleic Acid Stains for Cell Viability/Cytotoxicity Assays

The principle behind dye exclusion assays is based on the premise that viable cells with healthy plasma membranes are impermeable to several polar dyes such as trypan blue or cell-impermeant nucleic acid stains. The basics of this method consist of mixing cells in suspension with an impermeable dye and utilizing a fluorescence microscope, microplate reader or flow cytometer to examine the number of stained cells against the total cell population. The number of stained cells will represent the percentage of dead cells in the entire population. Dye exclusion assays and other cytotoxicity assays can also be used to indirectly measure cell viability. Utilizing the same technique but comparing the number of unstained cells to the total number of cells will represent the percentage of viable cells in a sample.

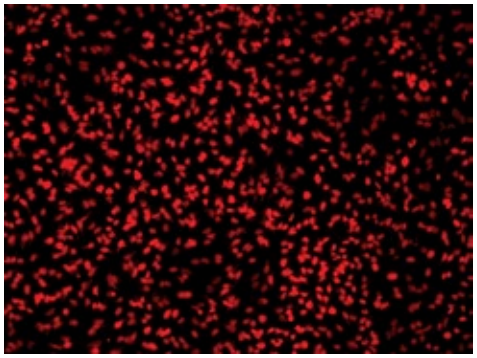


Figure 1.5 Fluorescence image of HeLa cells stained with Nuclear Red™ DCS1 (Cat# 17552) in a Costar black wall/clear bottom 96-well plate. Cells were monitored using a fluorescence microscope equipped with a Cy5 filter set.

Nuclear DCS1 Nucleic Acid Stains

Nuclear DSC1 dyes are a series of fluorescent and cell-impermeant nucleic acid stains. These dyes penetrate compromised membranes of dead cells and upon association with DNA generate significantly bright signals. These properties make Nuclear DSC1 dyes a simple and quantitative dead-cell indicator for use in fluorescence microscopy, flow cytometry or with fluorescence microplate readers.

Table 4. Cell-impermeant Nuclear DCS1 nucleic acid stains that can be used as a dead cell indicator.

Catalog #	Product Name	Ex/Em (nm)
17548	Nuclear Blue™ DCS1	350/461
17550	Nuclear Green™ DCS1	503/526
17551	Nuclear Orange™ DCS1	528/576
17552	Nuclear Red™ DCS1	642/660

Propidium Iodide

Propidium Iodide (PI) is a red fluorescent, cell-impermeable nucleic acid stain that belongs to the same chemical class as ethidium bromide (EtBr). Compared to ethidium bromide, PI exhibits a 20-30 fold fluorescence enhancement upon binding to nucleic

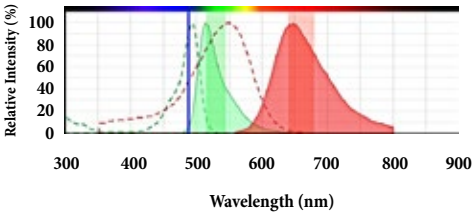


Figure 1.6 Excitation and emission spectrum of Calcein UltraGreen™ AM (Cat# 21905, Green) and 7-AAD (Cat# 17501, Red) illustrating the clear spectral separation that enables simultaneous visualization of live and dead cells in a population. Both Calcein UltraGreen™ AM and 7-AAD are efficiently excited by the 488 nm argon-ion laser. Calcein UltraGreen™ AM can be visualized with a FITC filter set. 7-AAD can be visualized with a Cy5 filter set.

Table 5. Available dimeric carbocyanine dyes that can be used as a dead cell indicator.

Catalog #	Product Name	Ex/Em (nm)
17571	TWO-PRO™-1	515/531
17572	TWO-PRO™-3	642/661
17575	DiTO™-1	514/535
17576	DiTO™-3	642/660
17580	DiYO™-1	491/509
17581	DiYO™-3	612/631

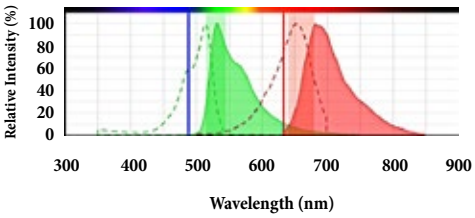


Figure 1.7 Excitation and emission spectrum of DiTO™-1 (Cat# 17576, Green) and Nuclear Red™ LCS1 (Cat# 17542, Red) illustrating the clear spectral separation that enables two-color cell viability and cytotoxicity assays. DiTO™-1 is efficiently excited by the 488 nm argon-ion laser and is visualized with a FITC filter set. Nuclear Red™ LCS1 is efficiently excited by the 633 nm helium-neon laser and is visualized with a Cy5 filter set.

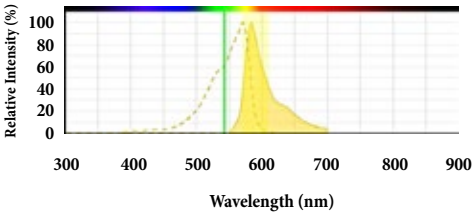


Figure 1.8 Excitation and emission spectrum of resazurin (Cat# 15700). Resazurin can be efficiently excited by the 543 nm green helium-neon laser and is visualized with a Cy3/TRITC filter set.

acids. Since PI has a relatively large Stokes Shift it can be used in combination with fluorescein-based indicators such as Calcein AM for the multicolor analysis of live and dead cells in a population. Since PI can be excited by the 488 nm argon laser line it can also be used for detecting and sorting dead cells by flow cytometry.

7-Aminoactinomycin D (7-AAD)

7-AAD is a cell-impermeant fluorescent indicator that can be used to identify non-viable cells. Upon association with DNA, 7-AAD emits at a wavelength of 647 nm when excited by the 488 nm argon laser. This makes it suitable for multiplexing assays in combination with other 488 nm-excited indicators such as Calcein AM. 7-AAD can be used in fluorescence microscopy, confocal laser-scanning microscopy, and flow cytometry.

Dimeric Cyanine Dyes

DiTO™, DiYO™ and TWO-PRO™ dyes are a series of cell-impermeant dimeric carbocyanine dyes that can be used as a dead cell indicator or a nuclear counterstain. These dyes are non-fluorescent in the absence of nucleic acids. Upon association with DNA, DiTO™, DiYO™ and TWO-PRO™ dyes generate bright fluorescence signals with extinction coefficients much greater than that of DNA-bound propidium iodide.

DiTO™, DiYO™ and TWO-PRO™ dyes generate strong and selective staining in cultured cells as well as in paraffin sections. These dyes in combination with other cell-permeant nucleic acid stains such as Nuclear Red™ LCS1 dyes can be used for two-color cell viability and cytotoxicity assays.

Fluorogenic Oxidation or Reduction Probes for Cell Viability/ Cytotoxicity Assays

The production of reactive oxygen species (ROS) is a normal by product of cellular metabolism, and in healthy cells occurs at a controlled rate. Metabolically active cells can oxidize or reduce reagents such as resazurin or tetrazolium salts, this can be used to assess cell viability as well as overall cell health.

Resazurin

Resazurin is a water-soluble, redox-sensitive dye that has been broadly used as a fluorescent indicator of cell viability in various cytotoxicity and proliferation assays. The reduction of resazurin to resorufin correlates with the number of viable cells in a population. This feature is widely used for assessing cell viability, cytotoxicity and proliferation. The level of reduction can be quantified by measuring the absorbance ratio of 570 nm and 600 nm, or by measuring fluorescence using a Cy3/TRITC filter set (Ex/Em= 571/585 nm). The colorimetric or fluorimetric signal generated is proportional to the number of viable cells in the sample. Resazurin may be added at any time during the culture period and its non-toxic properties keeps cells intact, which permits for parallel analyses. When measuring cell proliferation using resazurin it is recommended to add it during the log phase of growth.

Dihydrofluoresceins and Dihydrorhodamines

Fluorescein and rhodamine dyes can be chemically reduced to colorless, non-fluorescent "dihydro" dyes. Oxidation by reactive oxygen species returns dihydro-dyes back to the parent dye and restores its fluorescent properties. Because reactive oxygen species production is a characteristic of live but not dead cells, these fluorogenic oxidation probes can be used for assessing viability:

- Dihydrofluorescein diacetate and 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) both require hydrolysis by intracellular esterases and oxidation to restore fluorescent properties. Dihydrofluorescein diacetate and DCFH-DA both generate green fluorescence when excited by the 488 nm argon laser.
- Dihydrorhodamine 123 (DHR 123) is oxidized directly to mitochondrial stain rhodamine 123, which is generates green fluorescence when excited by the 488 nm argon laser.

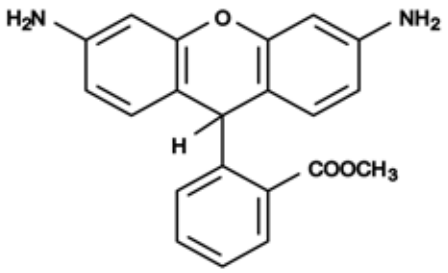


Figure 1.9 Chemical structure for Dihydrorhodamine 123 *CAS 109244-58-8*.

Tetrazolium Salts

Tetrazolium salts can be used for detecting redox potential of cells to assess viability and cytotoxicity. ReadiUse™ WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) is a water-soluble tetrazolium salt used for measuring cell metabolic activity. At neutral pH, cell-impermeant WST-8 is extracellularly reduced to a water-soluble orange formazan dye by a series of complex cellular mechanisms that occur primarily at the cell surface. This bio-reduction is dependent upon the glycolytic NADPH production of viable cells. The level of reduction can be quantified by measuring absorbance at 460 nm. The amount of formazan dye generated directly correlates to the number of viable cells in a sample. WST-8 is very stable and can tolerate long incubation periods (24 to 48 hours) due to its low cytotoxicity.

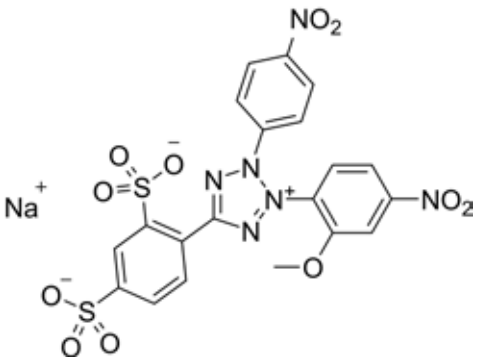


Figure 1.10 Chemical structure for ReadiUse™ WST-8 *50 mM aqueous solution*.

Potentiometric Dyes & Acidiotropic Probes for Cell Viability/ Cytotoxicity Assays

Viable cells contain an enormous amount of ion pumps and channels. These transmembrane proteins are responsible for maintaining intracellular ion concentration and transmembrane potentials which are essential for many vital cellular processes. When a cell dies it ceases to maintain its transmembrane potential and ion gradients. Changes in potential and ion gradients can be monitored utilizing potentiometric dyes, acidiotropic stains, ion indicators and pH probes to indicate cell viability.

Potentiometric Dyes

We provide a selection of potentiometric dyes for monitoring transmembrane potential gradients including rhodamine 123 and methyl and ethyl esters of tetramethylrhodamine (TMRM and TMRE). These positively charged rhodamine dyes selectively accumulate in the mitochondria of viable cells in an amount proportional to membrane potential. Rhodamine 123 can be used in combination with propidium iodide for two-color viability assessment by flow cytometry.

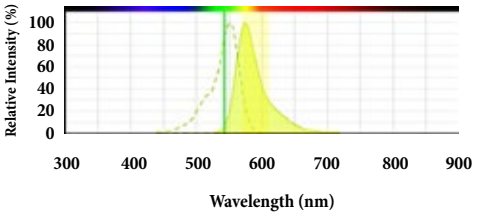


Figure 1.11 Excitation and emission spectrum of TMRM (Cat# 22221). TMRM can be efficiently excited by the 543 nm green helium-neon laser and is visualized with a Cy3/TRITC filter set.

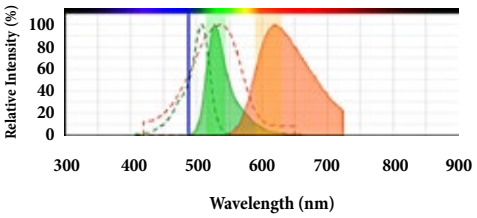


Figure 1.12 Excitation and emission spectrum of Rhodamine 123 (Cat# 22210, Green) and propidium iodide (Cat# 17517, Red) illustrating the clear spectral separation that enables two-color cell viability assessment by flow cytometry. Both Rhodamine and propidium iodide are efficiently excited by the 488 nm argon-ion laser. Rhodamine 123 can be visualized with a FITC filter set. Propidium iodide can be visualized with a Texas Red filter set.

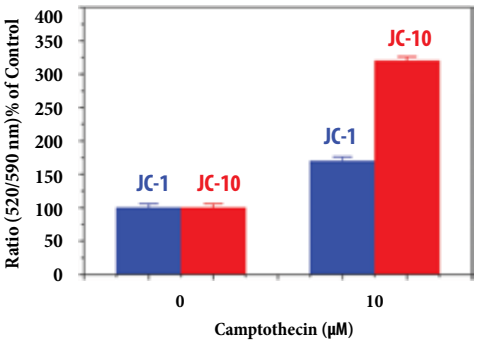


Figure 1.18 Campotothecin-induced mitochondria membrane potential changes were measured with JC-10™ and JC-1 in Jurkat cells. After Jurkat cells were treated with camptothecin (10 μM) for 4 hours, JC-1 and JC-10™ dye loading solutions were added to the wells and incubated for 30 minutes. The fluorescent intensities for both J-aggregates and monomeric forms of JC-1 and JC-10™ were measured at Ex/Em = 490/525 nm and 490/590 nm with NOVOstar microplate reader (BMG Labtech).

Fluorescent indicators such as JC-10™ can be used to monitor mitochondrial membrane potential. JC-10™ is superior and more convenient than JC-1 due to its higher sensitivity and improved water solubility. At low membrane potentials mitochondrial accumulation of JC-10™ is low and it will exist as a monomer. When excited by the 488 nm argon laser, monomeric JC-10™ fluoresces green. In viable cells with higher membrane potentials the accumulation of JC-10™ increases causing the formation of red-fluorescent J-aggregates. The capacity to make ratiometric measurements utilizing JC-10™ makes it practical for monitoring cell health.

Acidotropic Stains

Acidic organelles such as lysosomes contain membrane-bound proton pumps. In viable cells, these pumps maintain the organelles' relatively low pH by pumping protons (H+ ions) from the cytosol into the lysosomes. LysoBrite™ reagents are a series of fluorogenic probes that selectively accumulates in lysosomes of live cells via the lysosomal pH gradient. Upon entering lysosomes, LysoBrite™ reagents exhibit significantly enhanced fluorescence signals. The resulting fluorescence can be used to assess the percentage of viable cells in a population.

Product Ordering Information For Cell Viability and Cytotoxicity Assay Reagents

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
17501	7-AAD [7-Aminoactinomycin D] *CAS 7240-37-1*	1 mg	546	647
22007	Calcein blue, AM *CAS 168482-84-6*	1 mg	360	445
21902	Calcein Deep Red™	1 mg	646	659
22010	Calcein Deep Red™ acetate	1 mg	646	659
22009	Calcein Orange™ diacetate	1 mg	525	550
21900	Calcein Red™ AM	1 mg	560	574
21908	Calcein UltraBlue™ AM	10x50 ug	360	445
21905	Calcein UltraGreen™ AM	10x50 ug	496	524
22002	Calcein, AM *CAS 148504-34-1*	1 mg	495	515
22003	Calcein, AM *UltraPure grade* *CAS 148504-34-1*	1 mg	495	515
22021	CFDA [5-(and 6)-Carboxyfluorescein diacetate] *Mixed isomers*	100 mg	494	521
15203	Dihydrofluorescein diacetate [Fluorescin diacetate] *CAS 35340-49-9*	25 mg	490	514
15206	Dihydrorhodamine 123 *CAS 109244-58-8*	10 mg	507	529
22020	FDA [Fluorescein diacetate] *CAS 596-09-8*	1 g	494	521
22200	JC-1	5 mg	515	529
22204	JC-10 *Superior alternative to JC-1*	5x100 uL	510	525
22642	LysoBrite™ Blue	500 Tests	433	480
22646	LysoBrite™ Deep Red	500 Tests	596	619
22643	LysoBrite™ Green	500 Tests	445	503
22641	LysoBrite™ NIR	500 Tests	636	650
22644	LysoBrite™ Orange	500 Tests	542	556
22645	LysoBrite™ Red	500 Tests	575	597
17515	Propidium iodide *CAS 25535-16-4*	25 mg	535	617
15707	ReadiUse™ WST-8 *50 mM aqueous solution*	1 g	460	N/A
15700	Resazurin, sodium salt *CAS 62758-13-8*	100 mg	571	585
22210	Rhodamine 123 *CAS 62669-70-9*	25 mg	507	529
22220	TMRE [Tetramethylrhodamine ethyl ester] *CAS#: 115532-52-0*	25 mg	549	574
22221	TMRM [Tetramethylrhodamine methyl ester] *CAS#: 115532-50-8*	25 mg	549	573

Cell Viability and Cytotoxicity Assay Kits

The following section discusses our cell viability and cytotoxicity kits. These colorimetric, fluorimetric and chemiluminescent assay kits are reliable, easy to use and include all the essential components and a robust protocol for assessing cell viability and cytotoxicity.

Live or Dead™ Cell Viability Assay Kits

Our Live or Dead™ Cell Viability kits provide a simple and robust method for assessing cell viability and cytotoxicity of adherent or non-adherent cells. This kit employs two probes, a fluorogenic esterase substrate and a cell-impermeant DNA-binding dye, for a two-color discrimination of live cells from dead cells in a population. The fluorogenic esterase substrate is cell-permeant. Once inside cells, non-specific esterases hydrolyze the substrates generating a strong fluorescent signal proportional to the number of viable cells. The DNA-binding dye is polar and therefore impermeant to viable cells with intact membranes. It can only pass through the compromised membranes of dead cells and upon association with DNA fluoresces.

The reagents in our Live or Dead™ Cell Viability kits can be simultaneously added to the cell suspension and do not require any wash steps prior to analysis. Each assay can be readily adapted for us in flow cytometry, fluorescence microscopy or fluorescence microplate reader, and is suitable for high-throughput screening.

Live or Dead™ Fixable Dead Cell Staining Kit

The Live or Dead™ Fixable Dead Cell Staining Kits employ amine-reactive dyes to assess viability of mammalian cells by flow cytometry. In viable cells, the fluorescent dye's reactivity is restricted to cell-surface amines resulting in low fluorescence intensity. In dead cells or cells with compromised membranes, the dye reacts with intracellular free-amines and cell-surface amines generating an intense fluorescence signal. The difference in fluorescence intensity between the dead and live cell populations is ~100-500 folds and can be preserved after fixation. Because our single-color assays are designed to use one channel of a flow cytometer it enables multiparametric analysis with other dyes.

Cell Meter™ Cell Viability Assays

The Cell Meter™ Cell Viability Assay kits utilize our CytoCalcein™ dyes for a simple, single-color fluorescence assessment of cell viability in adherent or non-adherent cells. CytoCalcein™ dyes are hydrophobic compounds that readily permeate live cells. Once inside cells, non-specific esterases hydrolyze CytoCalcein™ dyes to generate strong fluorescence intensity which is proportional to the number of viable cells. Cell Meter™ Cell Viability assays are robust and sensitive without bearing any optical interference (a common problem associated with Alamar Blue based-assays). These kits are

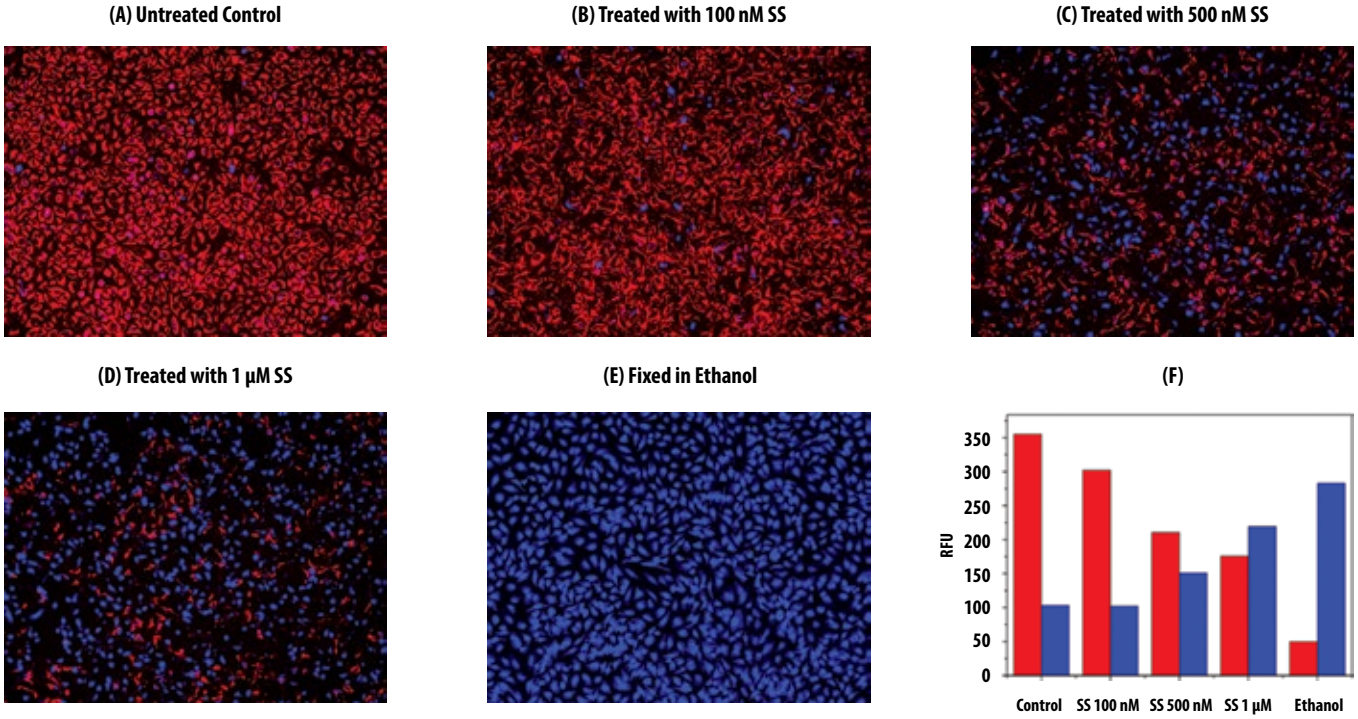


Figure 2.1 Fluorescence images of HeLa cells labeled with Live or Dead™ Cell Viability Assay Kit (Cat#22788). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 0-1 µM staurosporine (SS) at 37 °C for 4 hours (A-D), or fixed in ethanol (E), then incubated with dye -loading solution for 1 hour. The fluorescence signal was measured using a fluorescence microscope with TRITC filter for viable cells (Red) and DAPI filter for necrotic cells (Blue), respectively. (F) The corresponding fluorescence signal were measured using a FlexStation® microplate reader (Molecular Devices) with bottom read mode at Ex/Em =540/590 nm (cutoff=570 nm, Red) and Ex/Em= 360/450 nm (cutoff= 420 nm, Blue), respectively.

well-suited for use with fluorescence microplate readers and for high-throughput screening applications.

Cell Explorer™ Live Cell Labeling Kits

Our Cell Explorer™ Live Cell Labeling kits provide a convenient and robust method for assessing cell viability and cytotoxicity by fluorescence microscopy. Each kit includes all the necessary components and a robust labeling protocol for staining and quantifying both adherent and non-adherent cells in under two hours.

Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Cell Detection Kit

Our Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Cell Detection Kit employs a three-probe system to simultaneously monitor apoptotic, necrotic and healthy cells with a fluorescence microscope. Viable cells are detected and labeled using our cell-permeant dye Cellbrite™ Red (Ex/Em = 613/631nm). The initial and intermediate stages of apoptosis are characterized by the appearance of phosphatidylserine (PS) on the cell surface membrane. Cells undergoing apoptosis can be detected and labeled with PS sensor Annexin V-iFluor™ 488 (Ex/Em = 494/520 nm). Upon association with membrane exposed PS, Annexin V-iFluor™ 488 will fluoresce green. Dead or necrotic cells are detected and labeled with cell-impermeant nucleic acid stain, Nuclear Blue™ DCS1 (Ex/Em = 350/461 nm). The fluorescence signal generated by each probe is proportional to the number of apoptotic (green), necrotic (blue) or viable (red) cells, respectively.

Cell Meter™ Cell Cytotoxicity Assay Kits

Our Cell Meter™ Colorimetric Cell Cytotoxicity assay kit provides a robust and convenient method for monitoring cell viability. This kit employs a proprietary water-soluble and cell-permeant dye that changes its absorption spectra upon cellular reduction. The absorption ratio change is directly proportional to the number of viable cells. The Cell Meter™'s high sensitivity, use of non-radioactive material and no-wash method make it suitable for high-throughput screening of cell proliferation or cytotoxicity against a variety of compounds. This assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

A variety of parameters can be used for assessing cell viability such as measuring mitochondrial dehydrogenases (e.g. LDH) activity. The Cell Meter™ Fluorimetric Cell

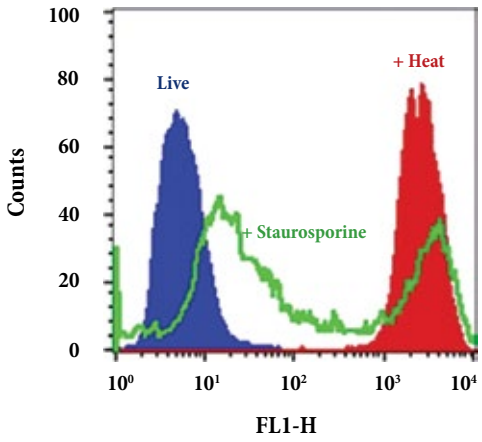


Figure 2.2 Detection of Jurkat cell viability by Live or Dead™ Fixable Dead Cell Staining Kit *Green Fluorescence* (Cat#22601). Jurkat cells were treated and stained with Stain IT™ Green. The cells were then fixed in 3.7% formaldehyde and analyzed by flow cytometry. Live (Blue solid peak), staurosporine treated (green line) and heat-treated (red solid peak) cells were distinguished with Ex/Em = 488/520 nm (FL1) channel. The live cell population is easily distinguished from the dead cell population, and nearly identical results were obtained using unfixed cells.

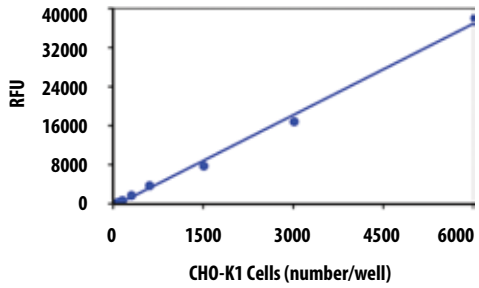


Figure 2.3 CHO-K1 cell number response was measured with Cell Meter™ Cell Viability Assay Kit. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ Green dye-loading solution for 1 hour at 37 °C. The fluorescence intensity was measured at Ex/Em = 490/ 525 nm with NOVOstar instrument (from BMG Labtech). The fluorescence intensity was linear (R^2 = 1) to the cell number as indicated.

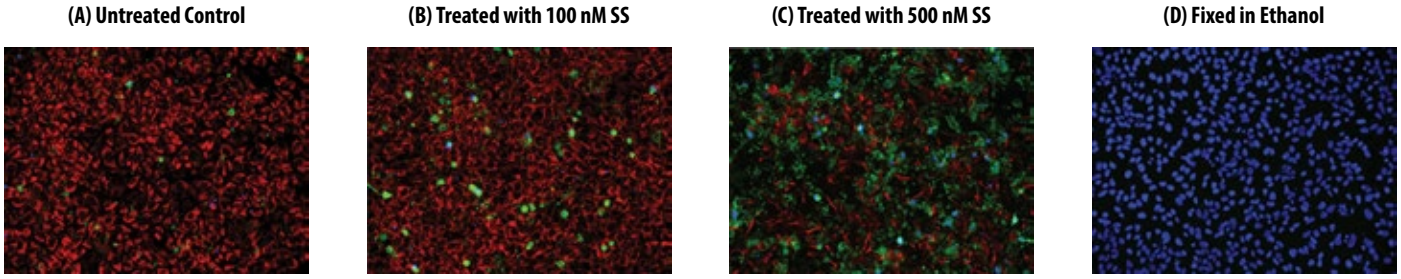


Figure 2.4 Fluorescence images of HeLa cells labeled with Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Detection Kit *Triple Fluorescence* (Cat#22846). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 0-500 nM staurosporine (SS) at 37°C for 4 hours (A-C), or fixed in ethanol (D), then incubated with triple fluorescence assay solution for 1 hour. The fluorescence signal was measured using a fluorescence microscope with a Cy5 filter for healthy cells (Red), FITC filter for apoptotic (Green) and DAPI filter for necrotic cells (Blue), respectively.

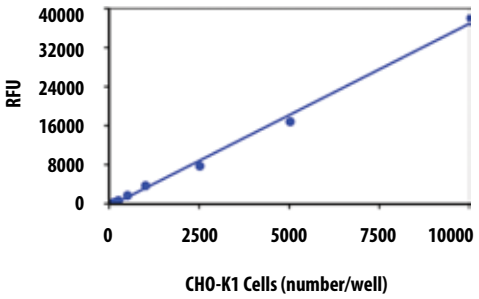


Figure 2.5 CHO-K1 cell number response was measured with Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit (Cat# 22781). CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 20 µL/well of Assay Solution (Component A) for 3 hours at 37 °C. The fluorescence intensity was measured at Ex/Em = 540/590 nm with NOVOstar instrument (BMG Labtech). The fluorescence intensity was linear (R^2= 0.998) to the cell number as indicated.

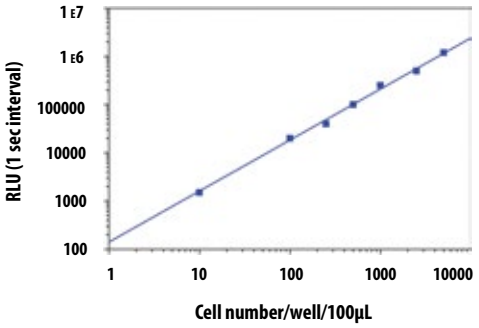


Figure 2.6 CHO-K1 cell number was measured with PhosphoWorks™ Luminescence ATP Assay Kit (Cat# 21610) on a 96-well white plate using a NOVOstar plate reader (BMG Labtech).

Cytotoxicity Kit provides a quick, simple and homogeneous assay for the fluorimetric detection of viable cells. This assay is based on the observation that oxidized non-fluorescent blue resazurin is reduced to the red-fluorescent dye resorufin by accepting an electron from the mitochondrial respiratory chain of live cells. The amount of resorufin produced correlates to the number of viable cells in a population. This assay can be performed in a convenient 96-well or 384-well microtiter-plate format. It is suitable for proliferating and non-proliferating cells, and can be used for both adherent and non-adherent cells.

PhosphoWorks™ Luminometric ATP Assay Kits

Adenosine Triphosphate (ATP) is a fundamental source of energy for many vital cellular processes including cellular energetics, metabolic regulation and cellular signaling. Because ATP rapidly degrades in the absence of living cells, it can be used for assessing cell viability in a population.

Our PhosphoWorks™ Luminometric Kits provide a fast, simple and homogenous bioluminescence method that can be used for the determination of cell viability, cytotoxicity and proliferation in mammalian cells by ATP detection. This kit employs two components: firefly luciferase and its substrate luciferin. Firefly luciferase is an enzyme that catalyzes the two-step oxidation of luciferin. In the presence of magnesium, luciferase catalyzes the reaction of luciferin, ATP and oxygen to yield an emission of light at 560 nm. When ATP is the limiting component, the intensity of light is proportional to the concentration of ATP. This assay is highly sensitive with the capacity to detect as low as 10 cells per well. It can be performed in a convenient 96-well or 384-well microtiter-plate format.

Product Ordering Information For Cell Viability and Cytotoxicity Assay Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
22606	Cell Explorer™ Live Cell Labeling Kit *Blue Fluorescence*	200 Test	360	445
22607	Cell Explorer™ Live Cell Labeling Kit *Green Fluorescence*	200 Test	495	515
22609	Cell Explorer™ Live Cell Labeling Kit *Red Fluorescence*	200 Test	646	659
22784	Cell Meter™ Cell Viability Assay Kit *Blue Fluorescence with 405 nm Excitation*	500 Tests	405	450
22785	Cell Meter™ Cell Viability Assay Kit *Blue Fluorescence*	500 Tests	360	445
22786	Cell Meter™ Cell Viability Assay Kit *Green Fluorescence*	500 Tests	495	515
22787	Cell Meter™ Cell Viability Assay Kit *NIR Fluorescence Optimized for Fluorescence Microplate Reader*	200 Tests	646	659
22783	Cell Meter™ Cell Viability Assay Kit *Red Fluorescence*	200 Tests	560	574
22780	Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit	1000 Tests	575	None
22781	Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit	1000 Tests	571	585
22846	Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Cell Detection Kit III *Triple Fluorescence Colors*	100 Tests	N/A	M/A
22789	Live or Dead™ Cell Viability Assay Kit *Green/Red Dual Fluorescence*	200 Tests	492; 540	515;620
22788	Live or Dead™ Cell Viability Assay Kit *Red/Blue Dual Fluorescence*	200 Tests	360; 550	450; 570
22600	Live or Dead™ Fixable Dead Cell Staining Kit *Blue Fluorescence*	200 Tests	353	442
22604	Live or Dead™ Fixable Dead Cell Staining Kit *Deep Red Fluorescence*	200 Tests	649	660
22501	Live or Dead™ Fixable Dead Cell Staining Kit *Green Fluorescence with 405 nm Excitation*	200 Tests	408	512
22601	Live or Dead™ Fixable Dead Cell Staining Kit *Green Fluorescence*	200 Tests	498	521
22502	Live or Dead™ Fixable Dead Cell Staining Kit *Orange Fluorescence with 405 nm Excitation*	200 Tests	398	550
22602	Live or Dead™ Fixable Dead Cell Staining Kit *Orange Fluorescence*	200 Tests	547	573
22599	Live or Dead™ Fixable Dead Cell Staining Kit *Red Fluorescence Optimized for Flow Cytometry*	200 Tests	523	617
22603	Live or Dead™ Fixable Dead Cell Staining Kit *Red Fluorescence*	200 Tests	583	603
21610	PhosphoWorks™ Luminometric ATP Assay Kit	100 Tests	N/A	560

Cell Proliferation and Cell Cycle Assay Reagents and Kits

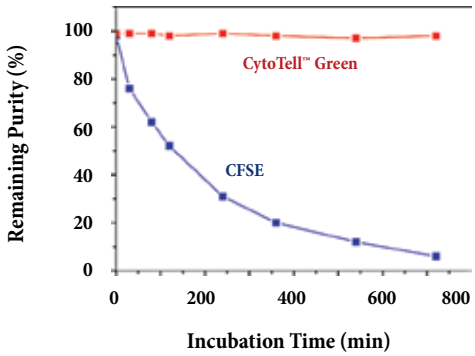


Figure 3.1 Stability comparison of CytoTell™ Green (Cat# 22253) and CFSE (Cat# 22022). 5 mM PBS working solutions of CytoTell™ Green and CFSE were monitored using HPLC (pH 7.2).

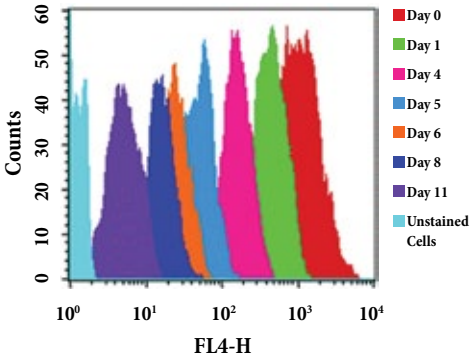


Figure 3.2 Cell tracking assay using CytoTell™ Red 650 (Cat# 22255). Jurkat cells (~ 2x106 cells/mL) were stained with CytoTell™ Red 650 (2 µM) on Day 0. The cells were passed serially at 1:1 ratio for 11 days. Fluorescence intensity was measured with FACSCalibur™ flow cytometer (BD, San Jose, CA) in FL4 channel on the day after passage. Successive generations were represented by different colors.

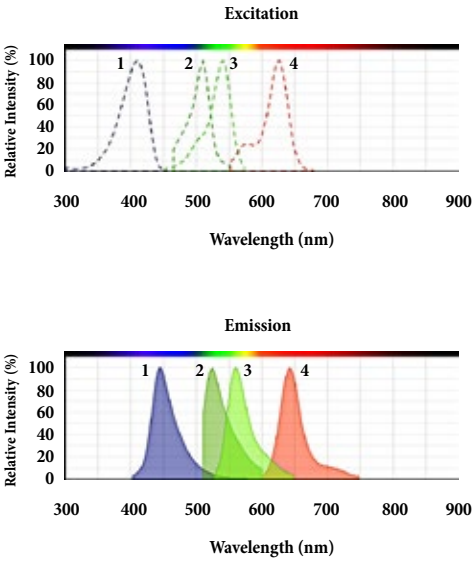


Figure 3.3 Excitation and emission spectrum of CytoTell™ dyes. **1)** CytoTell™ Blue (Cat#22251). **2)** CytoTell™ Green (Cat#22253). **3)** CytoTell™ Orange (Cat#22257). **4)** CytoTell™ Red 650 (Cat#22255).

Cell proliferation and cell cycle assays are designed to assess the growth of a cell population or detect the generation of daughter cells. When combined with cytotoxicity testing, proliferation assays can characterize the effects of novel pharmacological compounds on cell proliferation and cytotoxicity. This is an important area of investigation in cancer biology and drug-discovery research. The following section describes each of our cell proliferation and cell cycle assay reagents and kits.

Succinimidyl Ester Reagents for Cell Proliferation Assays

Carboxyfluorescein diacetate succinimidyl ester (CFSE) is a widely used cell proliferation indicator for determining cell growth. CFSE is a non-fluorescent cell-permeant probe that couples to intracellular and cell-surface proteins by reaction with free-amine groups. During cell division, CFSE labeling is equally distributed amongst daughter cells resulting in a fluorescence intensity half that of the parents. Each subsequent generation in a population of proliferating cells is characterized by a halving of cellular fluorescence intensity. This signal can be readily detected by a flow cytometer, fluorescence microplate reader or fluorescence microscope.

Since CFSE indiscriminately reacts with all amino groups it can be highly toxic to cells by disrupting the functionality of many critical intracellular proteins such as cell membrane GPCRs. Medium removal is required when using CFSE to image cells or analyze cells by flow cytometry. CFSE is known to react with medium components which can generated unwanted artifacts and contaminate data. Lastly, CFSE has spectral properties identical to GFP and FITC. This makes it difficult to use in multicolor applications with either GFP cell lines or FITC-labeled antibodies.

CytoTell™: Proliferation Dyes

Our CytoTell™ dyes were designed to address the aforementioned concerns associated with CFSE. Although functionally similar to CFSE, CytoTell™ dyes are suitable for multicolor analysis and are not hindered by the dye efflux drawback associated with CFSE. In addition, some of the CytoTell™ dyes do not require medium removal. CytoTell™ dyes are available in a variety of fluorescence emissions spanning the visible spectrum. CytoTell™ Red in particular can be used for long-term tracking. CytoTell™ Red stains cells evenly and during cell division is equally distributed amongst daughter cells. The successive halving of CytoTell™ Red's fluorescence intensity can be measured and visualize for up to eight generations. Additionally, some of the CytoTell™ dyes are fixable. Cells labeled with these dyes can be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives or saponin-based permeabilization buffers.

Cell Explorer™ Live Cell Tracking Kits

The Cell Explorer™ Live Cell Tracking kits provide a simple and convenient method for determining proliferating cells in a population. Each kit is designed to uniformly label live cells with a fluorescent tracking probe which is well retained within live cells for several generations. The labeling process is efficient and requires minimal hands-on time. Cell Explorer™ Live Cell Tracking kits are best-suited for use with a fluorescence microscope and flow cytometer. Additionally, we provide fixable Cell Explorer™ Live

Cell Tracking kits in green and red fluorescence. For further downstream investigation of cell surface or intracellular markers after proliferation.

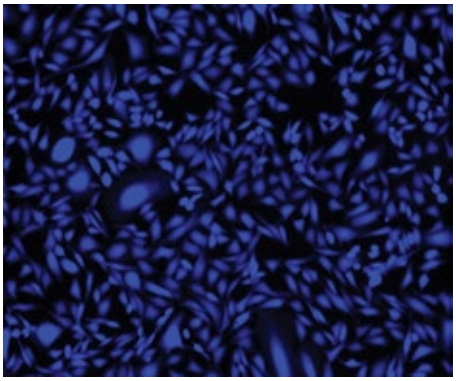


Figure 3.4 Fluorescence image of HeLa cells stained with Cell Explorer™ Live Cell Tracking Kit in a Costar black wall/clear bottom 96-well plate.

DNA Synthesis Cell Proliferation Reagents and Kits

Incorporation of thymidine analogs such as ^{3}H-thymidine is traditionally used for monitoring cell proliferation. Proliferating cells will incorporate this radioactive label into the newly synthesized DNA of replicating cells. To measure the radioactivity of DNA recovered from cells requires the use of a scintillation counter. Besides being tedious, the use of radioactive labels is hazardous and requires proper disposal of radioactive material.

5-bromo-2'-deoxyuridine (BrdU)

For a similar approach without the hazards and hassle of radioactive labels, consider 5-bromo-2'-deoxyuridine (BrdU). BrdU, which is incorporated in newly synthesized DNA, permits the indirect detection of proliferating cells with labeled anti-BrdU antibodies. Depending on the label used, enzyme or fluorophore, cell proliferation can be determined by colorimetric, fluorimetric or chemiluminescent detection. This technique is suitable for immunohistochemistry, ELISA, flow cytometry and high-throughput screening.

Bucculite™ dT Incorporation Cell Proliferation Fluorescence Imaging Kits

Our Bucculite™ dT Incorporation Cell Proliferation kits provides a superior alternative for detecting nascent DNA over ^{3}H-thymidine, BrdU and EdU incorporation techniques. These kits employ a fluorescently labeled nucleoside analog of thymidine, FOL-dT, which is incorporated into newly synthesized DNA during the replicating phase (S phase) of the cell cycle. After cell fixation, the incorporated FOL-dT is stained with MTA-iFluor™ 488 and visualized with a fluorescence microscope using either a FITC, TRITC, or Cy5 channel. This technique eliminates the use of anti-BrdU antibodies and permits for the direct detection of proliferating cells. Bucculite™ kits are convenient and do not use toxic materials such as radioactive labels or copper-catalyzed EdU.

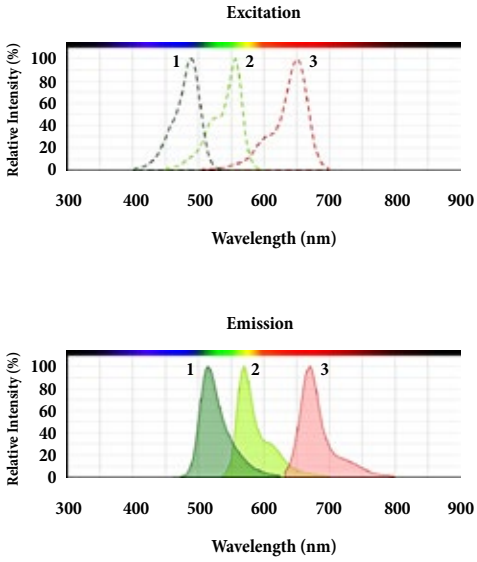


Figure 3.5 Excitation and emission spectrum of Bucculite™ dT Cell Proliferation kits. **1)** Bucculite™ dT with green fluorescence (Cat#22305). **2)** Bucculite™ dT with red fluorescence (Cat#22315). **3)** Bucculite™ dT with deep red fluorescence (Cat#22320).

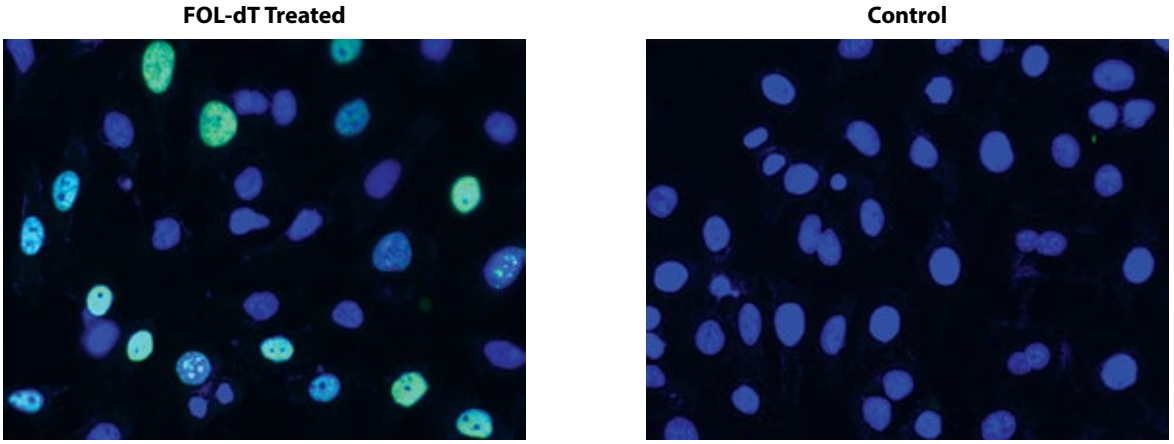


Figure 3.6 S-phase Hela cells were detected with Bucculite™ dT Incorporation Cell Proliferation Fluorescence Imaging Kit (Cat#22305). HeLa cells at 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with FOL-dT at 37 °C for 3 hours, and fixed with Methanol. After fixation, cells were stained with iFluor488-MTA for 30min in staining buffer, and then washed with PBS. 100uL Signal Enhancer were added to each well and the fluorescence images were visualized with FITC filter for S phase cells (Green) and nuclear for all cells (Blue) were costained with Hoechst 33342 for 30min and imaged with DAPI filter.

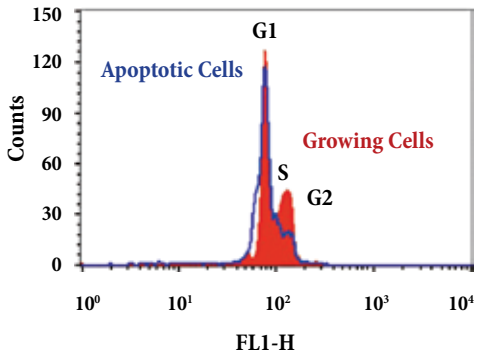


Figure 3.7 DNA profile in growing and camptothecin treated Jurkat cells. Jurkat cells were treated without (red) or with 20 µM camptothecin (blue) in a 37 oC, 5% CO2 incubator for about 8 hours, and assayed with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22841) according to the kit instruction. The fluorescence intensity of Nuclear Green™ LCS1 (Component A) was measured with a FACSCalibur™ flow cytometer using the FL1 channel. In growing Jurkat cells, nuclei stained with Nuclear Green™ LCS1 showed G1, S, and G2 phases (red). In camptothecin treated apoptotic cells (B), the fluorescence intensity of Nuclear Green™ LCS1 was decreased, and both S and G2 phases were diminished.

Bucculite™ proliferation assays are suitable for multiplexing applications with fluorescently labeled antibodies.

Cell Cycle Assays

Many parameters can be monitored for assessing proliferation and cell viability. In normal cells, DNA density varies depending on it progression through the cell cycle. The cell cycle consists of four sequential phases: G0/G1, S, G2, and M. DNA replication occurs during the S phase. This newly synthesized DNA is then equally distributed between two daughter cells. DNA content can then be measured using the appropriate fluorescent nucleic indicators which emit signals proportional to DNA mass.

Cell Meter™ Cell Cycle Assays

Our Cell Meter™ Fluorimetric Cell Cycle kits provide a convenient and simple method for monitoring cell proliferation in both permeabilized and fixed cells. They are designed to monitor cell cycle progression utilizing our proprietary nucleic acid stains. These cell-permeant dyes intercalate cellular DNA. Upon excitation, the fluorescence intensity generated is directly proportional to the DNA content. Flow cytometric analysis of these stained populations will reveal the percentage of cells in each of the cell cycle phases.

Product Ordering Information For Cell Proliferation and Cell Cycle Assay Reagents and Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
17030	BrdU [5-Bromo-2'-deoxyuridine] *CAS 59-14-3*	25 mg	N/A	N/A
22320	Bucculite™ dT Incorporation Cell Proliferation Fluorescence Imaging Kit *Deep Red Fluorescence*	200 Tests	649	665
22305	Bucculite™ dT Incorporation Cell Proliferation Fluorescence Imaging Kit *Green Fluorescence*	200 Tests	491	518
22315	Bucculite™ dT Incorporation Cell Proliferation Fluorescence Imaging Kit *Red Fluorescence*	200 Tests	559	565
22621	Cell Explorer™ Fixable Live Cell Tracking Kit *Green Fluorescence*	200 Tests	495	515
22625	Cell Explorer™ Fixable Live Cell Tracking Kit *Red Fluorescence*	200 Tests	575	600
22620	Cell Explorer™ Live Cell Tracking Kit *Blue Fluorescence*	200 Tests	403	445
22624	Cell Explorer™ Live Cell Tracking Kit *Deep Red Fluorescence*	200 Tests	637	650
22622	Cell Explorer™ Live Cell Tracking Kit *Orange Fluorescence*	200 Tests	528	541
22623	Cell Explorer™ Live Cell Tracking Kit *Red Fluorescence*	200 Tests	575	600
22841	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 Tests	503	526
22845	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Optimized for 405 nm Violet Laser Excitation*	100 Tests	401	459
22842	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 Tests	535	617
22022	CFSE [5-(and 6)-Carboxyfluorescein diacetate, succinimidyl ester] *CAS 150347-59-4*	25 mg	494	521
22251	CytoTell™ Blue	500 Tests	403	454
22252	CytoTell™ Blue	1000 Tests	403	454
22253	CytoTell™ Green	500 Tests	511	525
22254	CytoTell™ Green	1000 Tests	511	525
22257	CytoTell™ Orange	500 Tests	542	556
22258	CytoTell™ Orange	1000 Tests	542	556
22261	CytoTell™ Red 590	500 Tests	573	588
22262	CytoTell™ Red 590	1000 Tests	573	588
22255	CytoTell™ Red 650	500 Tests	628	643
22256	CytoTell™ Red 650	1000 Tests	628	643
22240	CytoTell™ UltraGreen	500 Tests	492	519
22241	CytoTell™ UltraGreen	1000 Tests	492	519

Alphabetical Index

Catalog Number Index

International Distributors

Austria:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Australia:
Assay Matrix Pty Ltd.
Email: info@assaymatrix.com
Website: http://www.assaymatrix.com

Life Research Pty Ltd.
Email: info@liferesearch.com
Website: http://www.liferesearch.com

Belgium:
Gentaur BVBA
Email: info@gentaur.com
Website: http://www.gentaur.com

Canada:
Cedarlane Laboratories Ltd.
Email: sales@cedarlanelabs.com
Website: http://www.cedarlanelabs.com

China:
Tianjin Biolite Biotech Co., Ltd
Email: info@tjbiolite.com
Website: http://www.tjbiolite.com

Croatia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Czech Republic:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

Denmark:
Nordic BioSite ApS
Email: info@nordicbiosite.dk
Website: http://www.nordicbiosite.dk

Estonia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Finland:
Nordic BioSite OY
Email: info@biosite.fi
Website: http://www.biosite.fi

France:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Interchim
Email: interchim@interchim.com
Website: http://www.interchim.com

Germany:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Hong Kong:
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com

Hungary:
IZINTA Trading Co., Ltd.
Email: baloghk@izinta.hu
Website: http://www.izinta.hu

Iceland:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

India:
Biochem Life Sciences
Email: info@bcls.in
Website: http://www.bcls.in

GenxBio Health Sciences Pvt. Ltd,
Email: sales@genxbio.com
Email: genxbio@gmail.com
Website: http://www.genxbio.com

Ireland:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

Israel:
ADVANSYS Technologies for Life Ltd.
Email: info@advansys.co.il
Website: http://www.advansys.co.il

Italy:
Space Import Export S.r.l.
Email: info@spacesrl.com
Website: http://www.spacesrl.com

Valter Occhiena S.r.l.
Email: vo@valterocchiena.com
Website: http://www.valterocchiena.com

Japan:
Cosmo Bio Co., Ltd.
Email: mail@cosmobio.co.jp
Website: http://www.cosmobio.co.jp

Nacalai Tesque, Inc.
Email: info@nacalaiusa.com
Website: http://www.nacalai.com

Wako Pure Chemical Industries, Ltd.
Email: labchem-tec@wako-chem.co.jp
Website: http://www.wako-chem.co.jp

Korea:
Cheong Myung Science Corporation
Email: cms@cmscorp.co.kr
Website: http://www.cmscorp.co.kr

Kimnfriends Corporation
Email: kimnfriends@hanmail.net
Website: http://www.kimnfriends.co.kr

Latvia and Lithuania:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Netherlands:
ITK Diagnostics BV
Email: info@itk.nl
Website: http://www.itk.nl

Norway:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Poland and Slovenia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Romania:
SC VitroBioChem SRL
Email: office@vitro.ro
Website: http://www.vitro.ro

Singapore and Other South Asian Countries:
BST Scientific Pte Ltd.
Email: info@bstsci.com
Website: http://www.bstsci.com

Slovakia:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

South American Countries and Regions:
Impex Comércio Internacional Ltda.
Email: impexcom@terra.com.br
Website: http://www.impexbrasil.com.br

Spain and Portugal:
Deltaclon S. L
Email: info@deltaclon.com
Website: http://www.deltaclon.com

Sweden:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

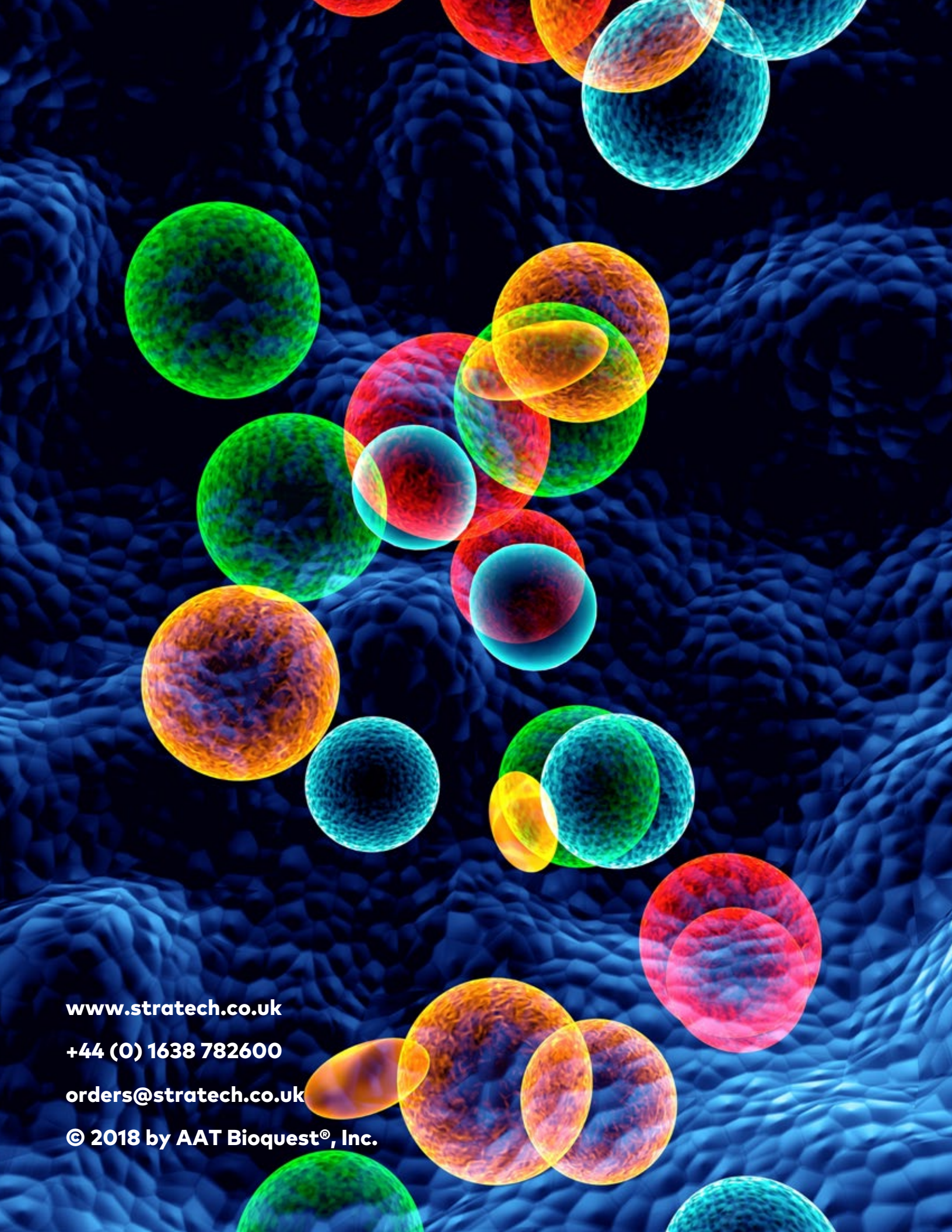
Switzerland:
LuBioScience GmbH
Email: info@lubio.ch
Website: http://www.lubio.ch

Taiwan:
Cold Spring Biotech Corp.
Email: csbiotech@csbiotech.com.tw
Website: http://www.csbiotech.com.tw

Rainbow Biotechnology Co., LTD.
Email: rainbow@rainbowbiotech.com.tw
Website: http://www.rainbowbiotech.com.tw

Turkey:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

United Kingdom:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk



www.stratech.co.uk

+44 (0) 1638 782600

orders@stratech.co.uk