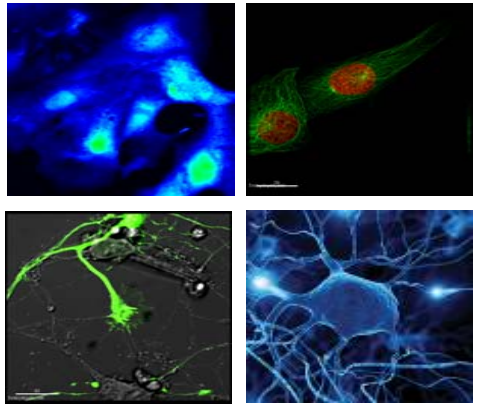

New Chapters from

The Molecular Probes® Handbook

Fluorescence Labeling and Detection Strategies in Cellular Biology

Daniel W. Beacham, PhD

Senior Staff Scientist, Research and Development

Molecular Probes, Cellular Imaging and Analysis

Daniel.Beacham@lifetech.com

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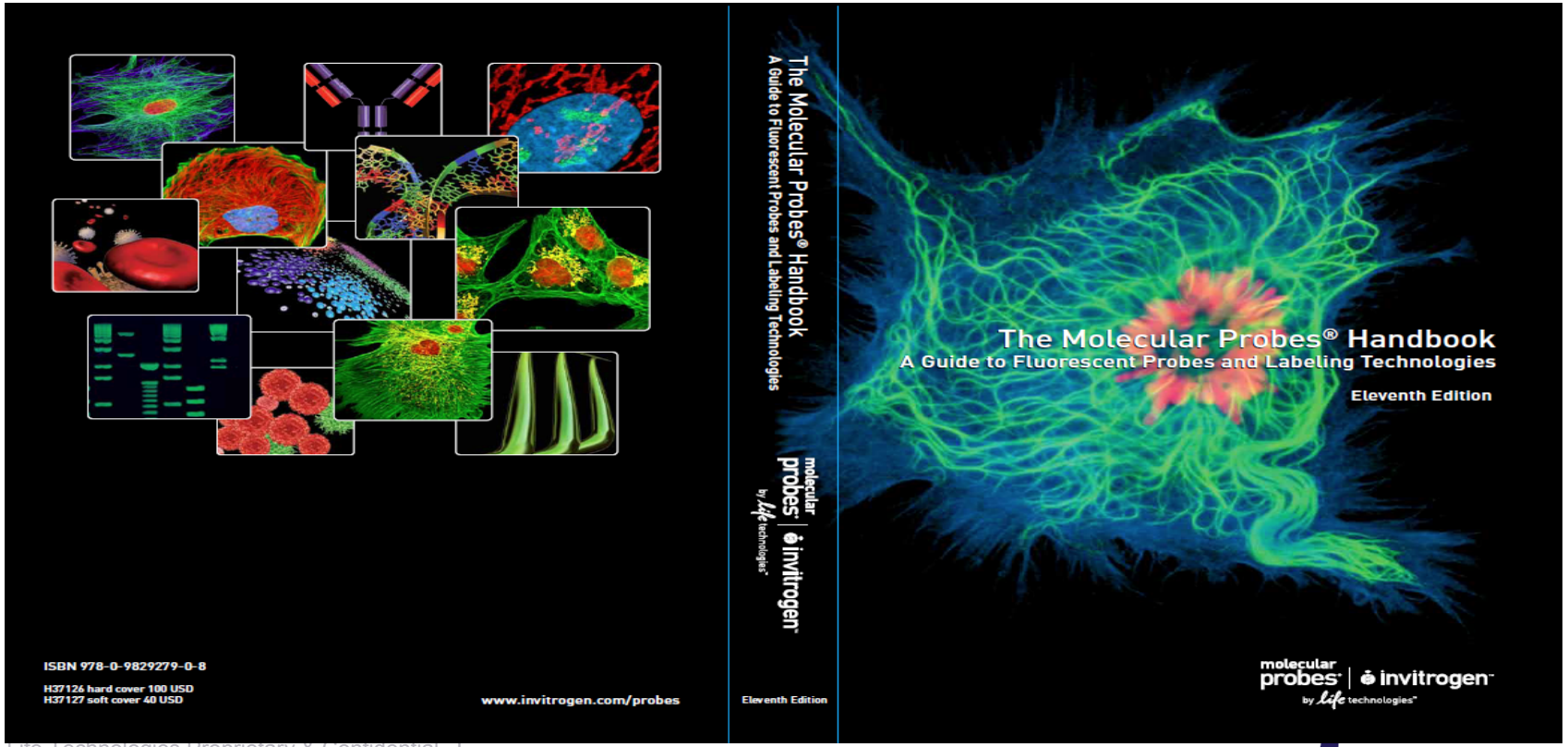
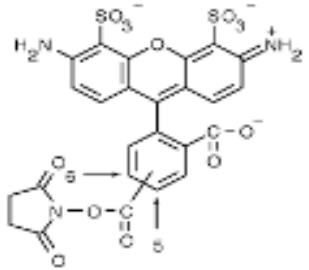
The Handbook on Fluorescence Detection



Dyes, Indicators and Affinity Labels

Fluorescent Dyes

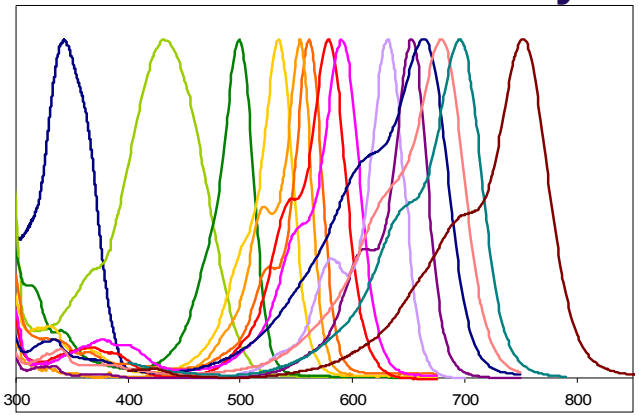


Alexa Fluor® 488

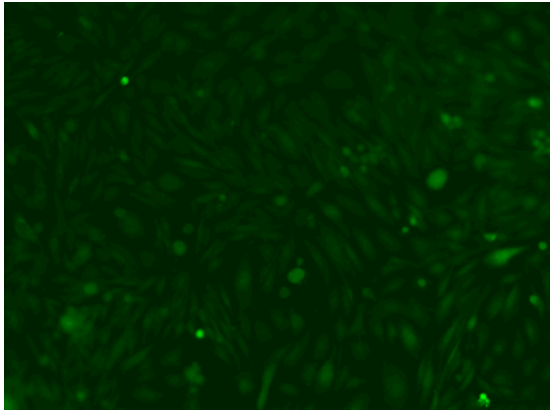
- MW – ~250 –1800 Da
- Emission λ: ~400–850+ nm
- Ext. coeff.: 20,000–250,000 $M^{-1}cm^{-1}$
- Designed for use in live and/or fixed cell applications, some are fixable

- Antibody & streptavidin conjugates
- Ion & voltage indicators
- Organelle stains
- Protein labeling
- Cell tracing
- Enzyme substrates

Alexa Fluor® Dyes



Fluo-4 Calcium Indicator



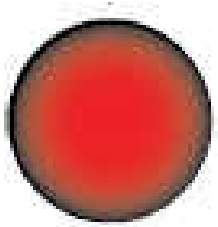
Fluorescent Proteins & Nanocrystals

Fluorescent Proteins

- MW: ~25,000-30,000 Da
- Emission λ: ~450–650 nm
- Ext. coeff.: 5,000–150,000 $M^{-1}cm^{-1}$
- Designed for use in live cell applications, fixable
- Genetically encoded

- Protein expression
- Living biosensors
- Addressable organelle and cellular landmarking
- DIY tagging, FRET assay, trafficking tools

Fluorescent QDot® Nanocrystals



- MW: Not applicable
- Emission λ: ~450–850 nm
- Ext. coeff.:140,000–8,000,000 $M^{-1}cm^{-1}$
- Primary use fixed cell applications, with limited live cell applications
- Photostable

- Antibody & streptavidin conjugates
- Cell tracing
- Vascular imaging
- Electron microscopy

Overview of Technologies

Imaging Toolbox

Indicator and Labeling Dyes

pHrodo™ pH sensor, Click-iT® chemistry

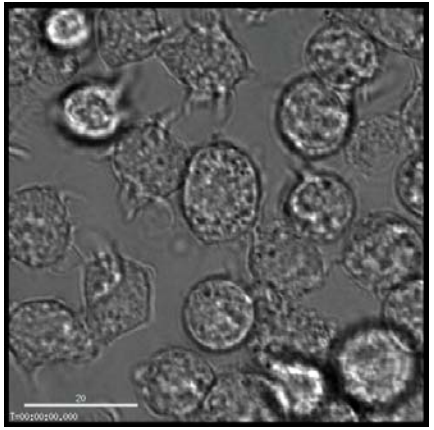
BacMam Fluorescent Proteins

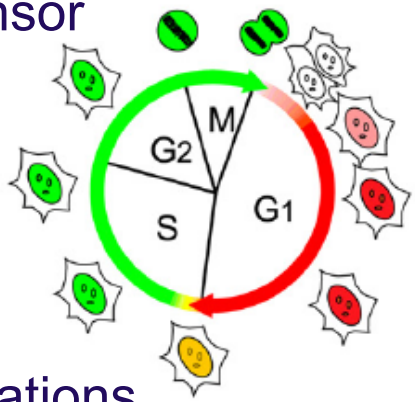
Targeted FPs and Biosensors

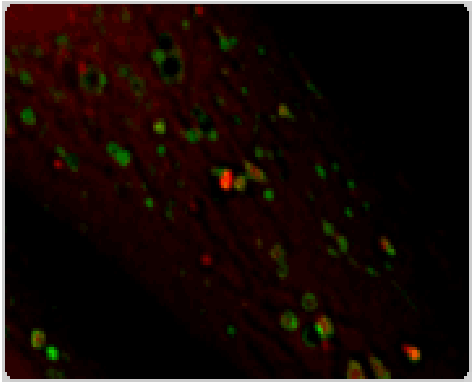
Rhod-3 AM Calcium Indicator

*CellEvent™ Caspase 3/7 Sensor

*CellROX™ Deep Red ROS Sensor







HCS ToolBox

HCS CellMask™ Stains

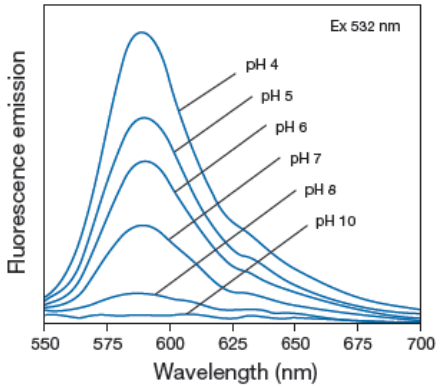
HCS Mitochondrial Health Kit

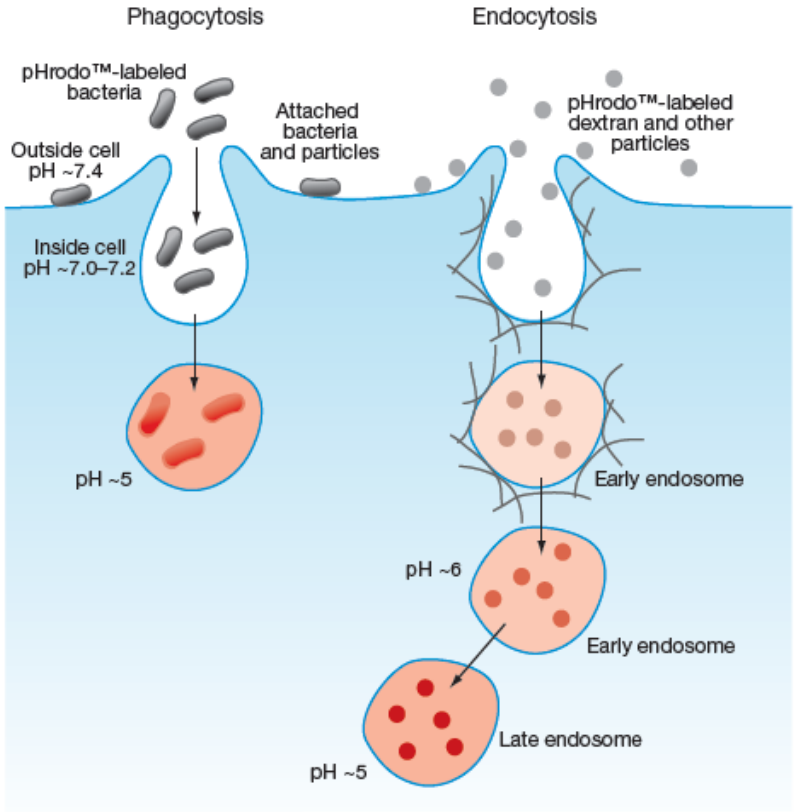
Click-iT® HCS Tools

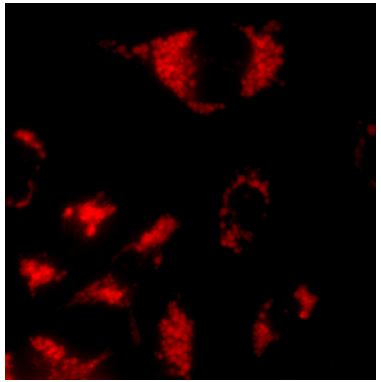
Mitotic Index Kit and HCS applications

life technologies™

Probing Compartmental pH with pHrodo™ Dyes







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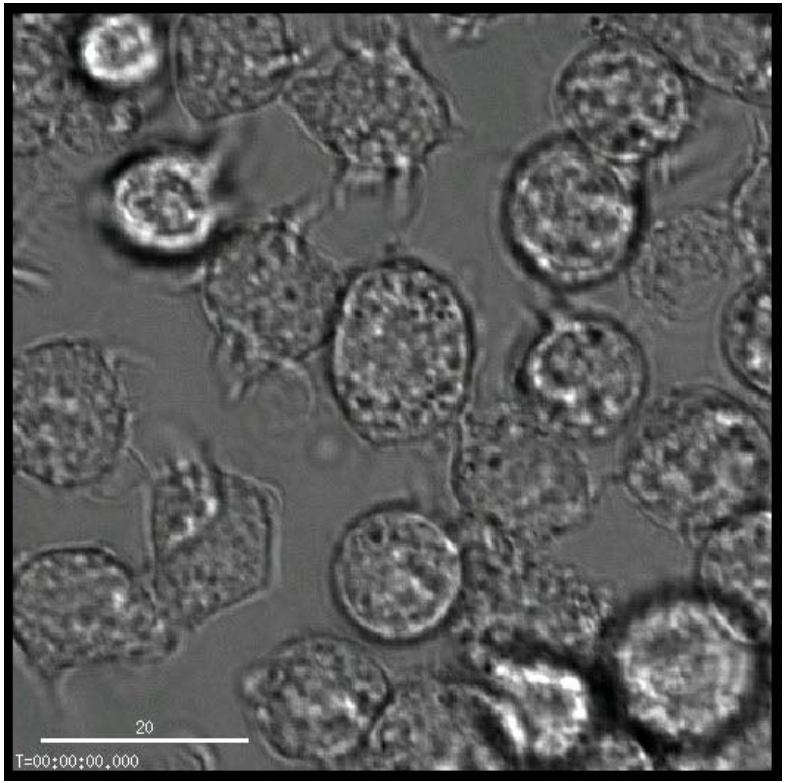
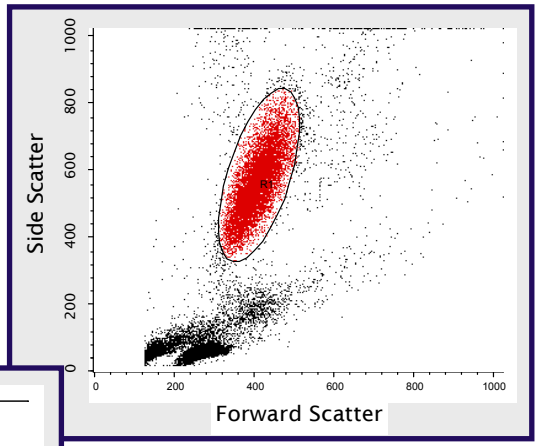
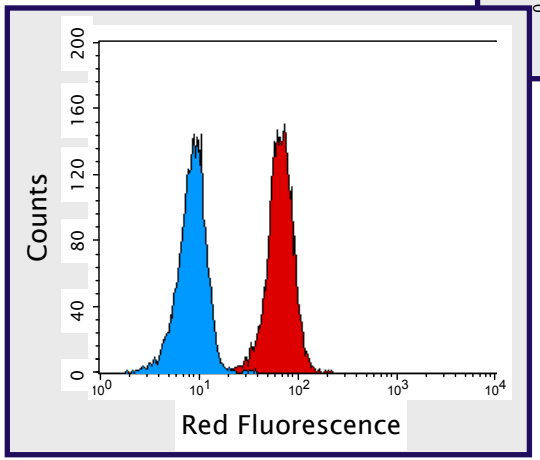
Phagocytic Index with pHrodo™ Dye

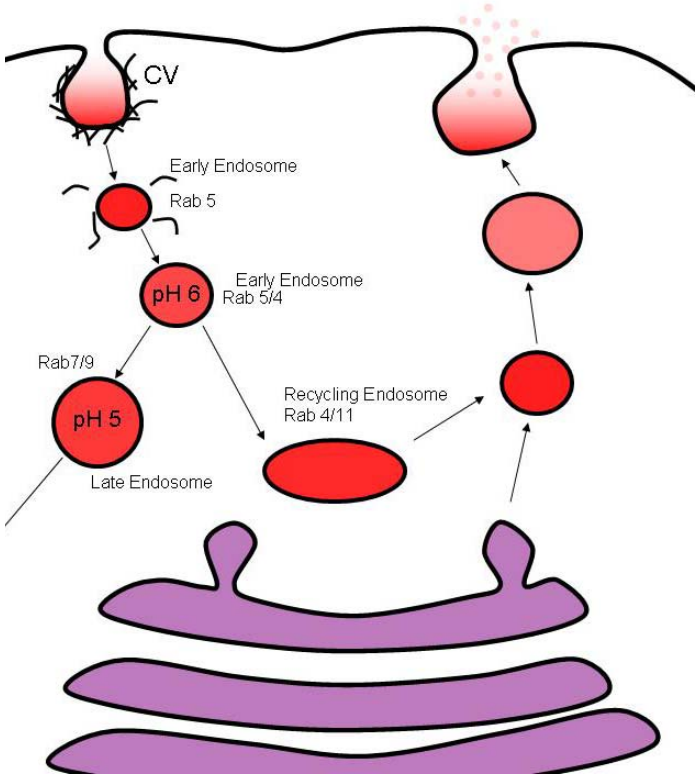
DeltaVision™ Core

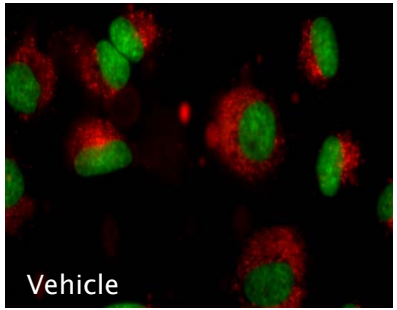


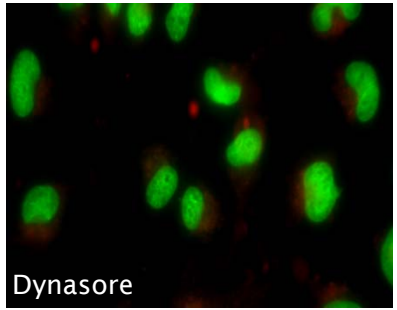


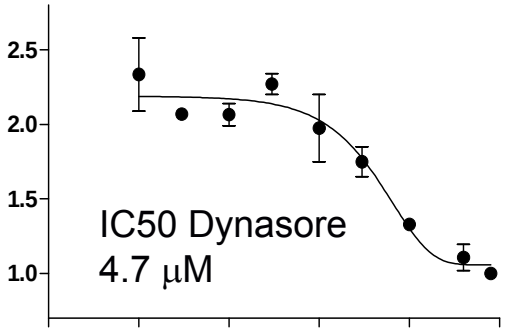


pHrodo™ Dextran for Endocytosis and Sorting





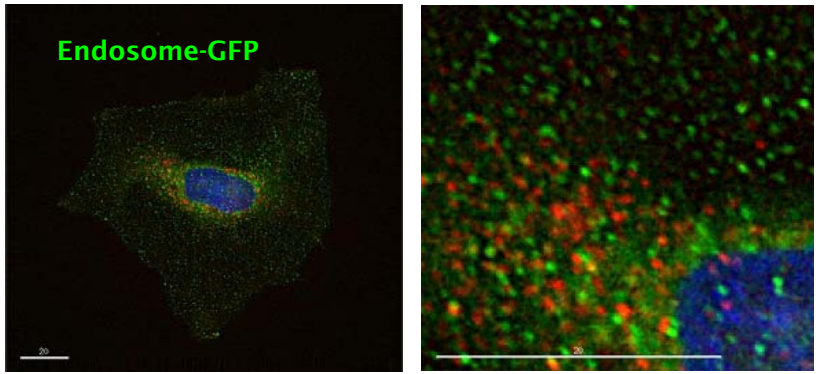




Endocytosis with pHrodo™ 10K-dextran

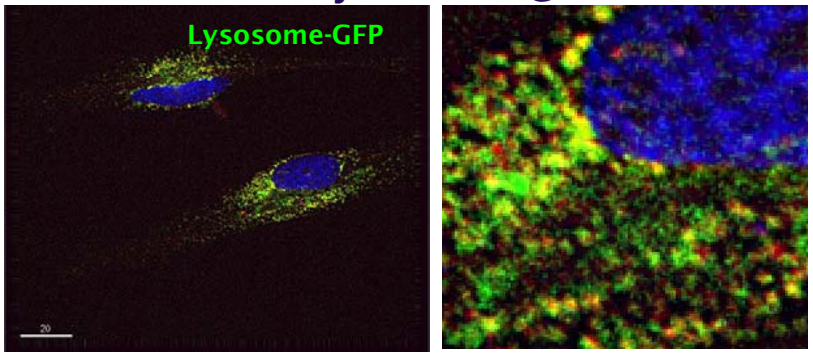
1)

Initial signal seen in endosomes @ 10 mins



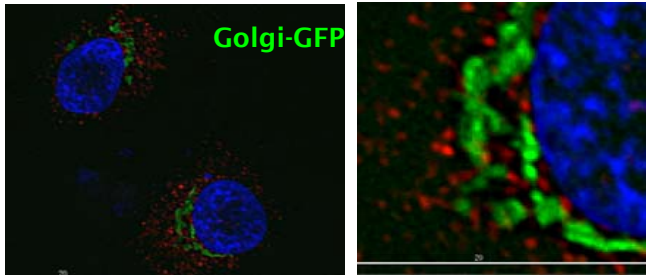
2)

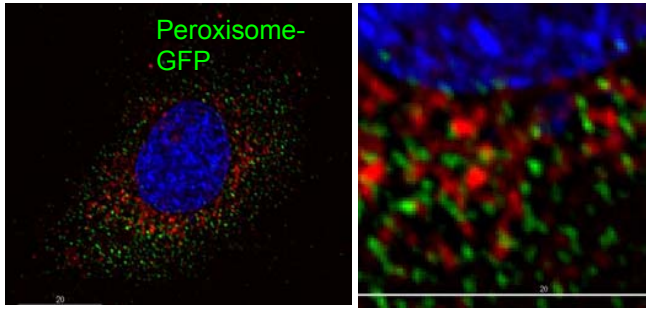
…Trafficked into lysosomes @ 30 mins



3)

Sorted away from Golgi and Peroxisomes





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pHrodo™ Dextran Multiplex

pHrodo™ Dextran conjugates are visible in endosomes and lysosomes

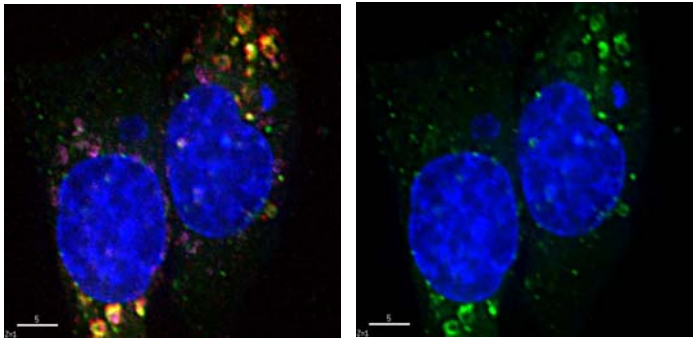
Transit through endosomes quickly
- *BacMam Endosome GFP
- *AF Transferrin Uptake

Emission is brighter in lysosomes than endosomes

Lysosomal pH is more acidic

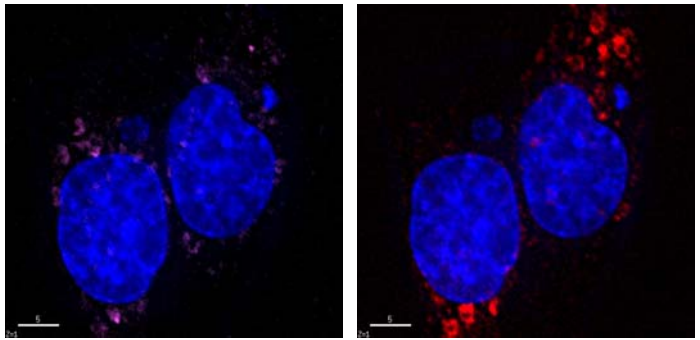
OVERLAY

CellLight™ Endosome-GFP

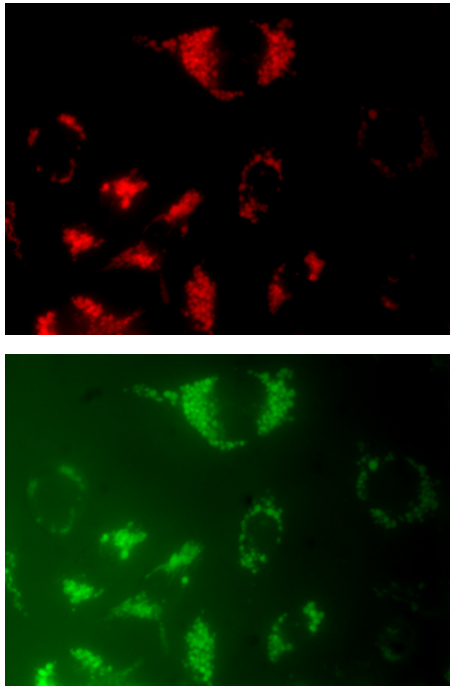


AF-647-TfN

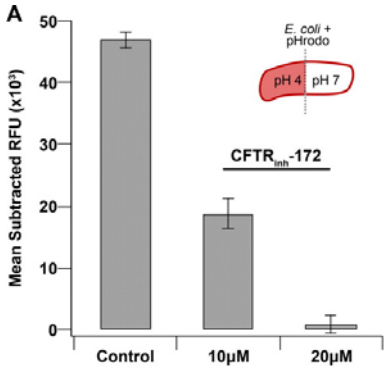
pHrodo™ Dextran

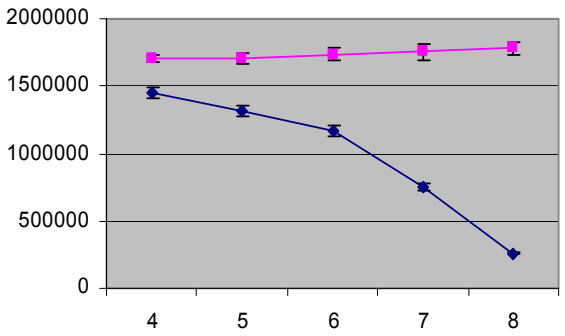


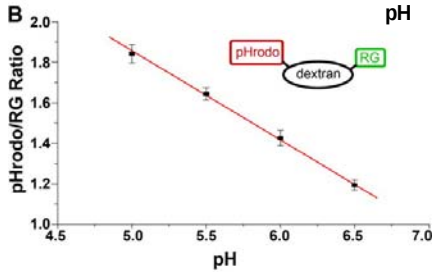
pHrodo™ Dextran Ratiometry

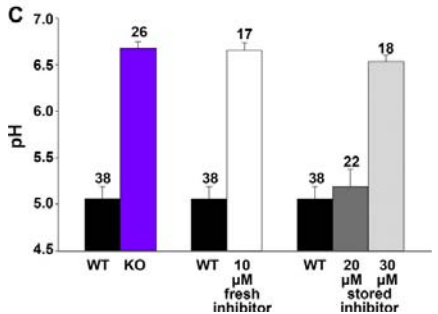


Concentration dependence of the CFTRinh-172-induced inhibition of acidification in phagosomes and lysosomes.









Disease-causing Mutations in the Cystic Fibrosis Transmembrane Conductance Regulator Determine the Functional Responses of Alveolar Macrophages Deriy et al. J. Biol. Chem. 2009;284:35926-35938

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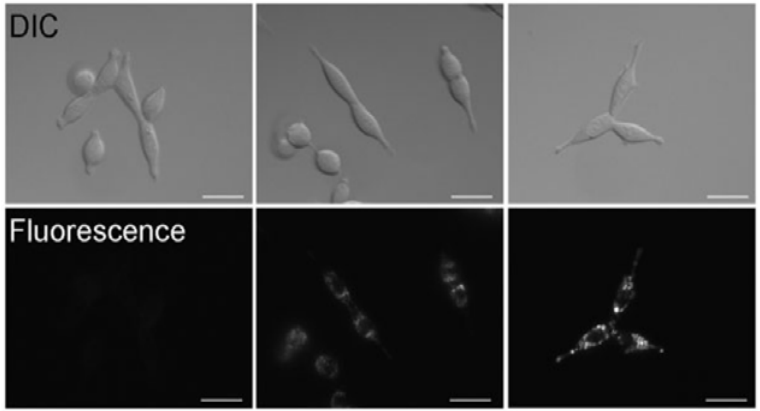
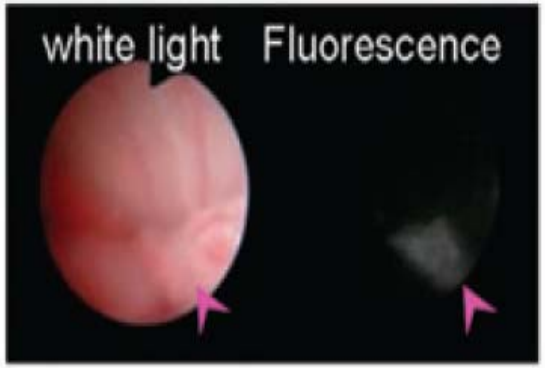
pHrodo™ Labeled Antibody Internalization

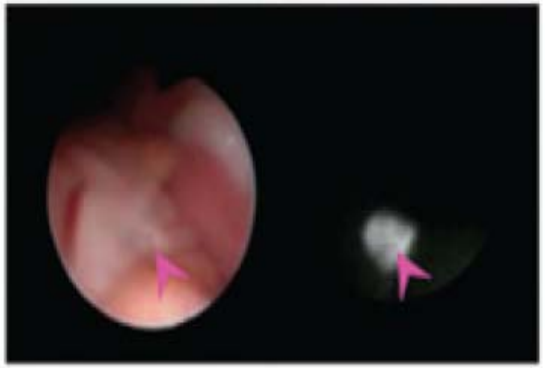
pHrodo SE Amine-Reactive dye for customized labeling

High sensitivity detection of cancer *in vivo* using a dual-controlled activation fluorescent imaging probe based on H-dimer formation and pH activation†

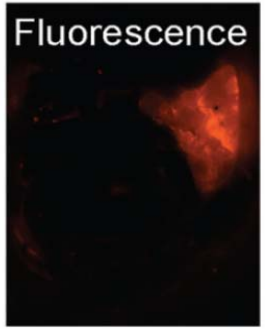
Mikako Ogawa, Nobuyuki Kosaka, Celeste A. S. Regino, Makoto Mitsunaga
Peter L. Choyke and Hisataka Kobayashi

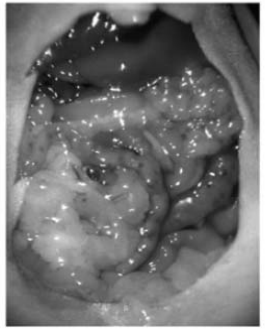


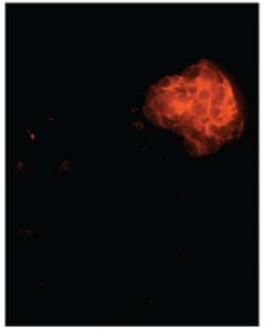




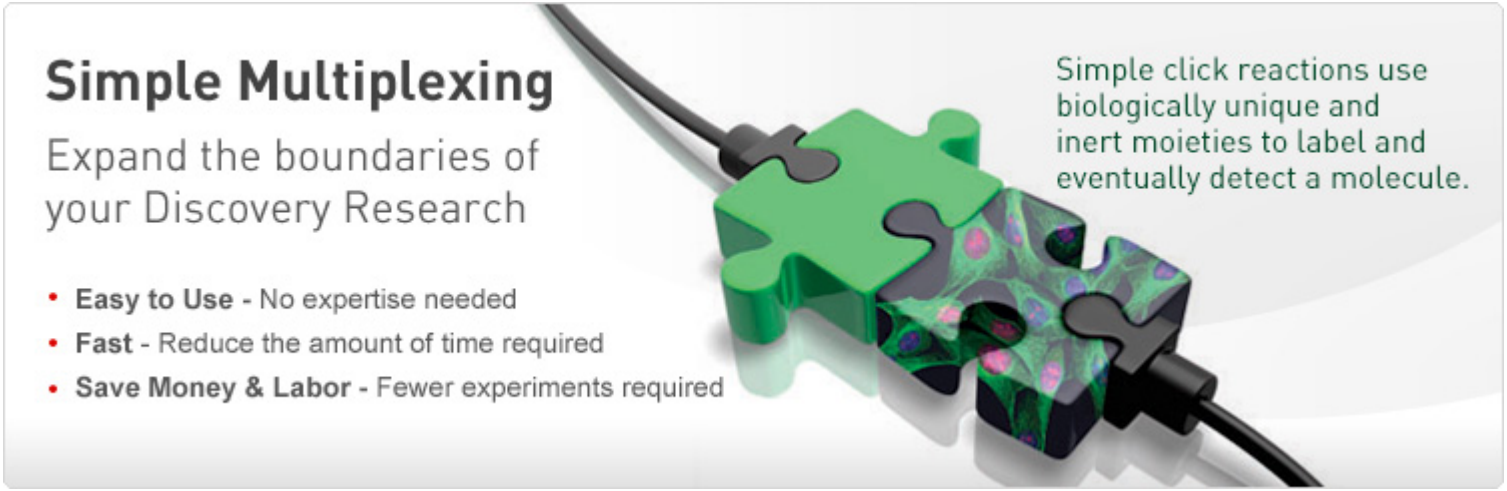
a 1hr 2hrs







Click-iT® Chemistry for Modular Detection



Nascent DNA Synthesis/ Cell Proliferation	Detection of DNA Strand Breaks	Nascent RNA Synthesis	Nascent Protein Synthesis

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What is Click-iT®?

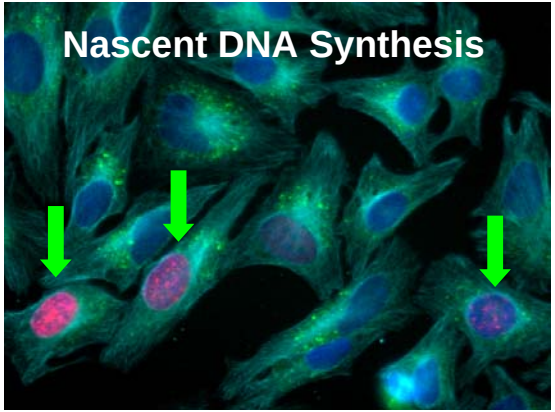
- Small, bio-orthogonal and "interchangeable" functional groups
- Chemoselective ligation reaction

Two-step procedure:

1) Label molecule of interest metabolically, enzymatically or chemically

2) Versatile detection, compatible with *any readout* (fluorescence, absorbance), on *any platform* (blot, gel, flow cytometer, imaging, mass spectrometer)

Copper catalyzed click reaction between an alkyne and an azide



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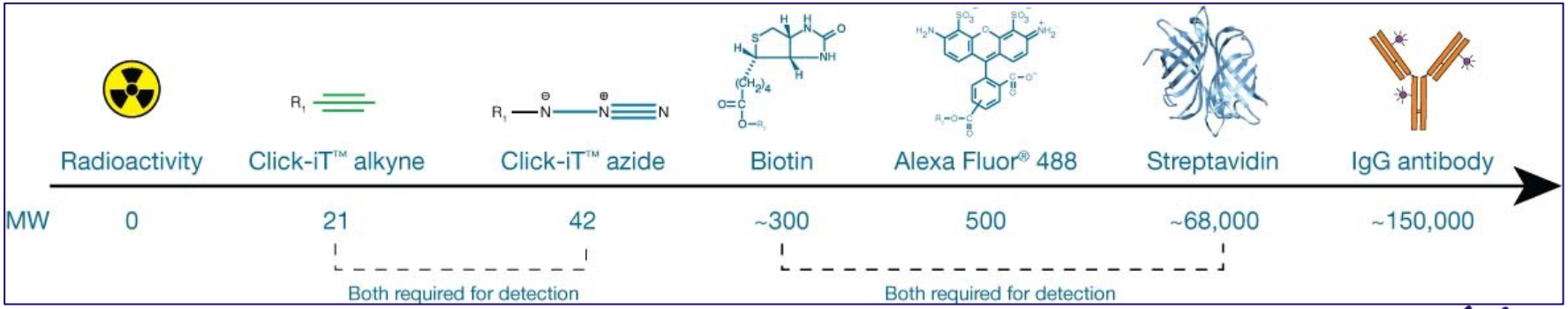
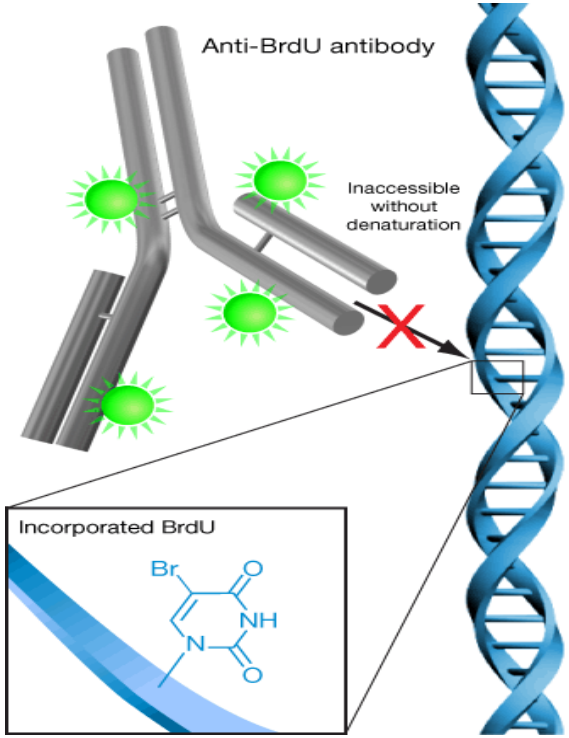
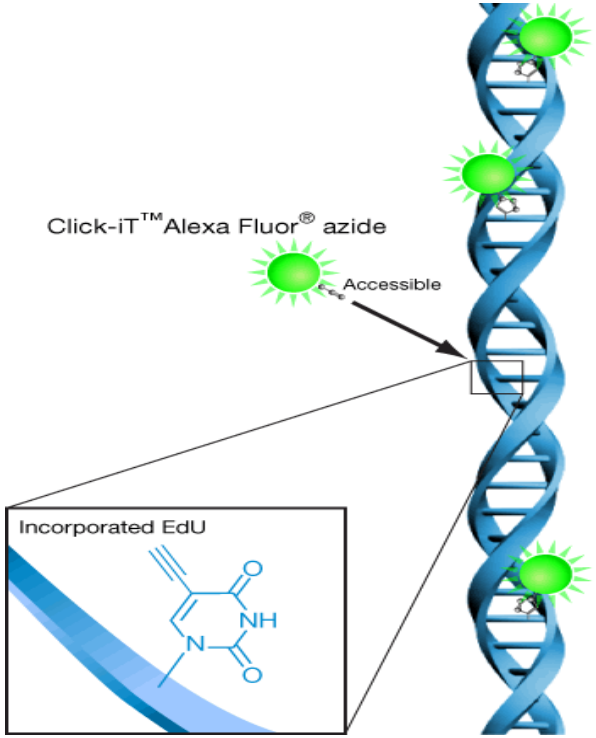
Click-iT® Chemical Biology



Click-iT® EdU: Proliferation on Single Cell Level



BrdU



Click-iT® EdU

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Incubate with EdU or BrdU, fix & permeabilize sample

Click chemistry EdU Protocol	
Click-iT™ detection reaction	30 minutes
Wash 2X	10 minutes
Nuclear counterstain	15 minutes
Wash 3X	15 minutes
Image	TOTAL TIME 70 minutes

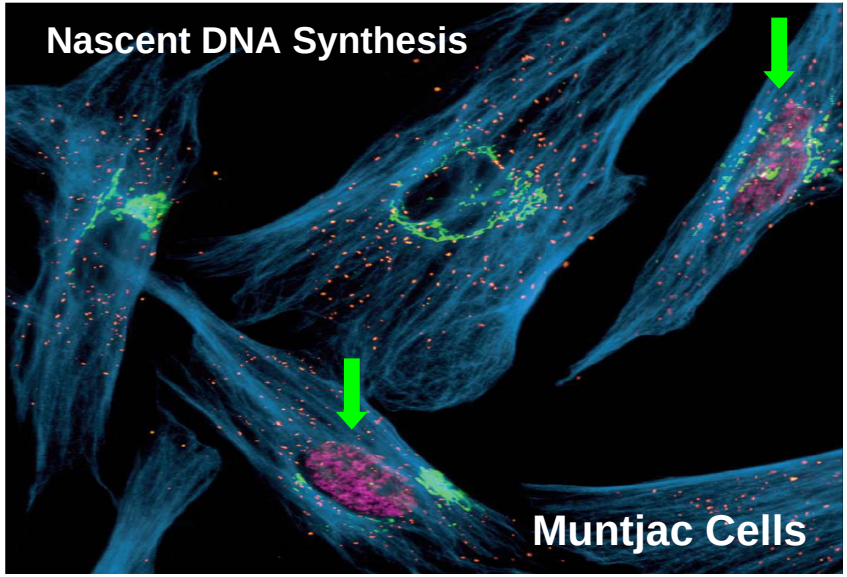
BrdU Protocol	
Wash 3X	15 minutes
HCl Denaturation	40 minutes
Neutralize	12 minutes
Wash 3X	15 minutes
Block	60 minutes
Anti-BrdU incubation	1-16 hours
Wash 3X	15 minutes
Secondary antibody incubation	120 minutes
Wash 3X	15 minutes
Nuclear counterstain	15 minutes
Wash 3X	15 minutes
Image	TOTAL TIME 21 hours

With Click chemistry

- **Measure proliferation in cells or tissue**
- **Time to complete: <2 hours**
- **Detect by fluorescence microscopy, flow cytometry or high-throughput imaging (HCS)**

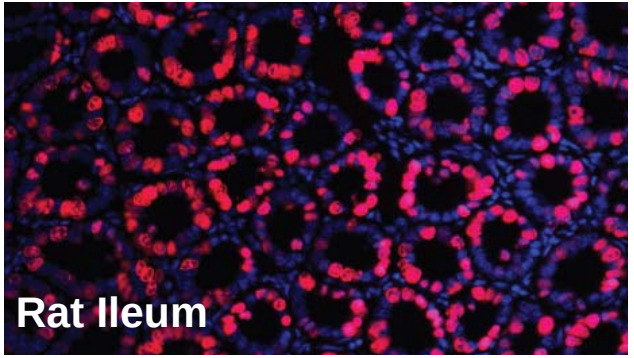
Intact Cell Morphology with Click-iT ® EdU

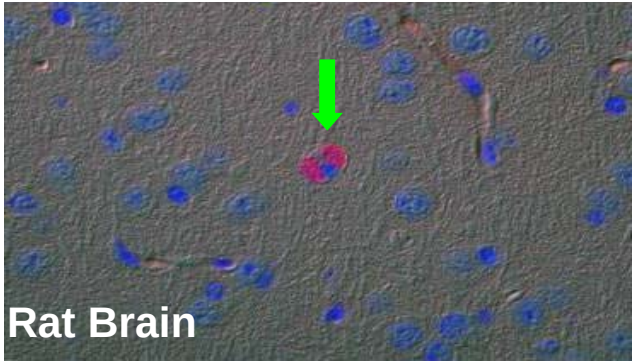
Cellular Label



Muntjac skin fibroblasts with Click-iT® EdU Alexa Fluor® 647 (purple), immuno-detection of tubulin (blue), golgi (green) and peroxisomes (orange).

Whole Animal





Salic, A. (2008) Proc Natl Acad Sci USA 105:2415–2420

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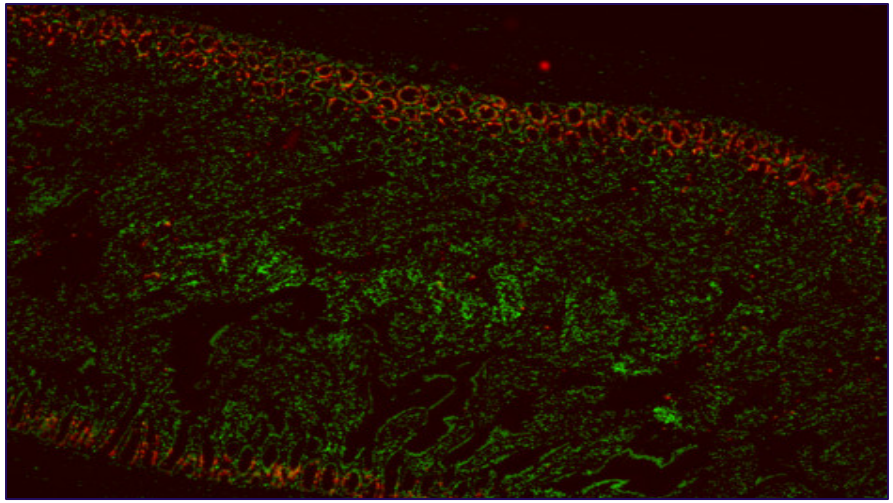
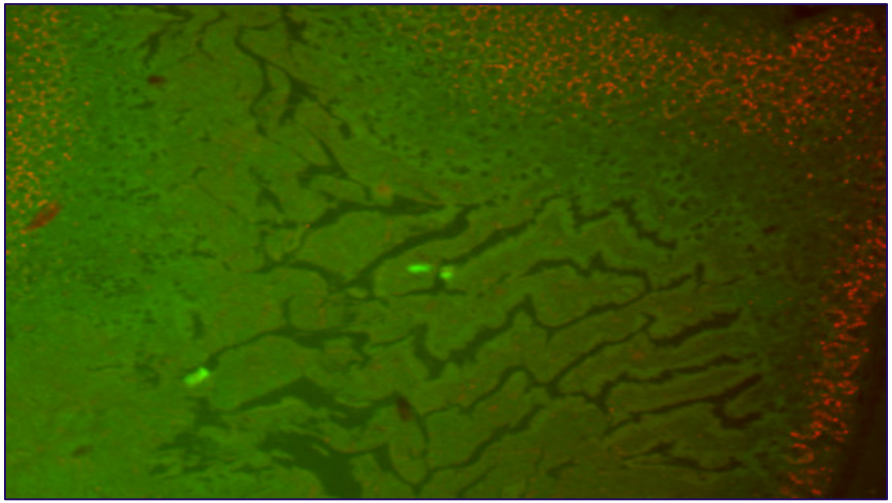
Click-iT® EdU vs BrdU detection in tissue sections

Click-iT® EdU protocol

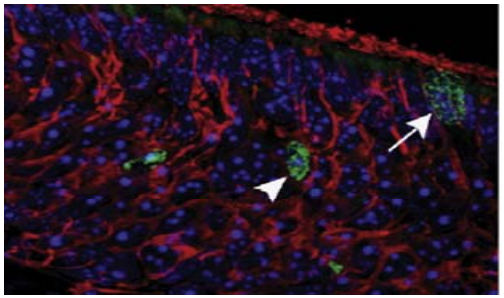


BrdU protocol

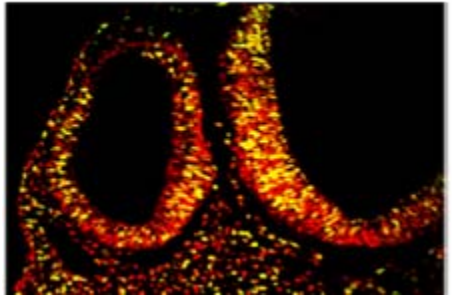


Green: Hoechst 33342. Red: EdU or BrdU detection with Alexa Fluor® 594 dye

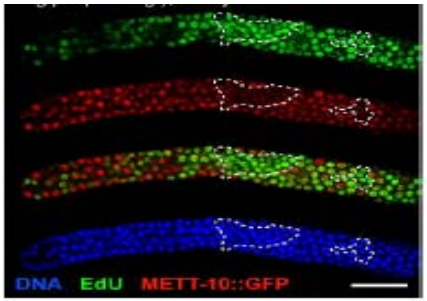
Click-iT® EdU Across Species (and Kingdoms)



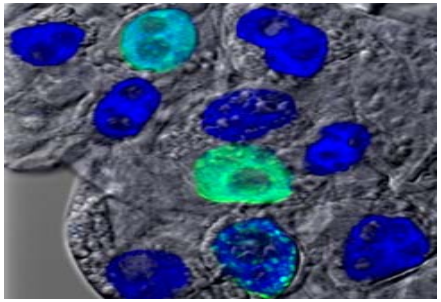
Click-iT® EdU labeling birth-dates differentiated cells in mouse olfactory epithelium F. Chehrehasa et al. J Neurosci Method (2009)



Neural tube and otocyst labeling with Click-IT® EdU in chick embryos. M. Warren et al. Dev. Dynamics (2009)



Proliferating germ cells in *C. elegans* with Click-iT® EdU. M. Dorsett et al. Genetics (2009)



Medicago sativa (alfalfa) suspension cultures labeled with Click-iT® EdU. Image courtesy of Ferhan Ayadin, Cellular Imaging Laboratory, Biological Research Center

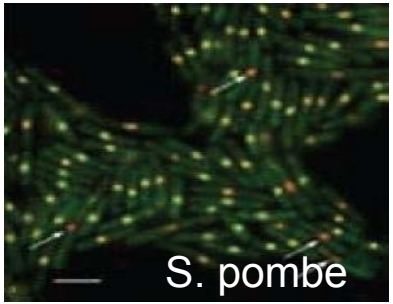


Image courtesy of Sarah A. Sabatinos, University of Southern California

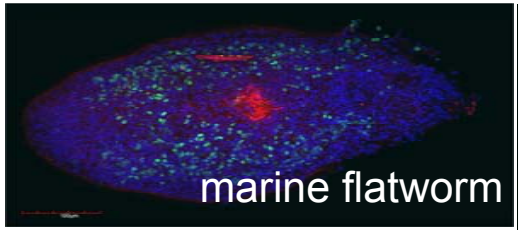


Image courtesy of Julian P.S. Smith III, Winthrop Microscopy Facility, Winthrop University

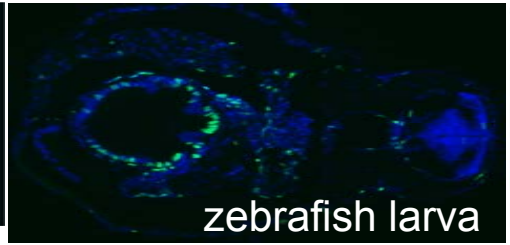
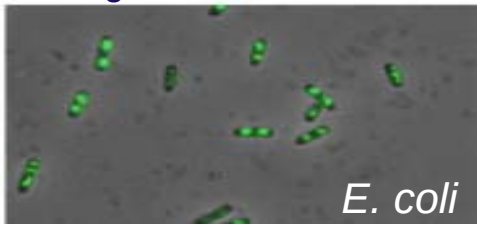


Image courtesy of Sarah Cheesman, University of Oregon



Methods (2009) 48:8-13

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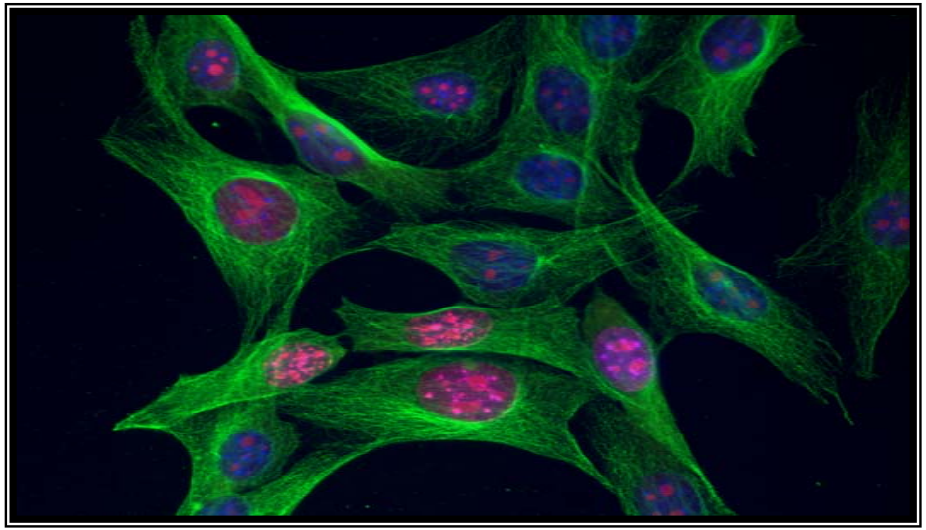
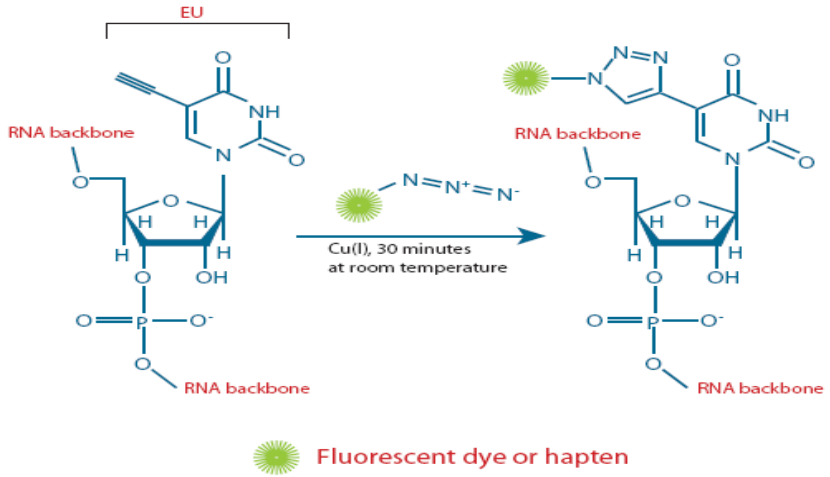
Click-iT® Based Detection of Nascent RNA Synthesis



NIH 3T3 cells incubated with 1 mM EU for 1 hr followed by click reaction with Alexa Fluor® 647 azide (magenta), immuno-detection of tubulin with Alexa Fluor® 488 secondary (green), and Hoechst nuclear counterstain (blue).

EU Assay (5-ethynyl uridine)



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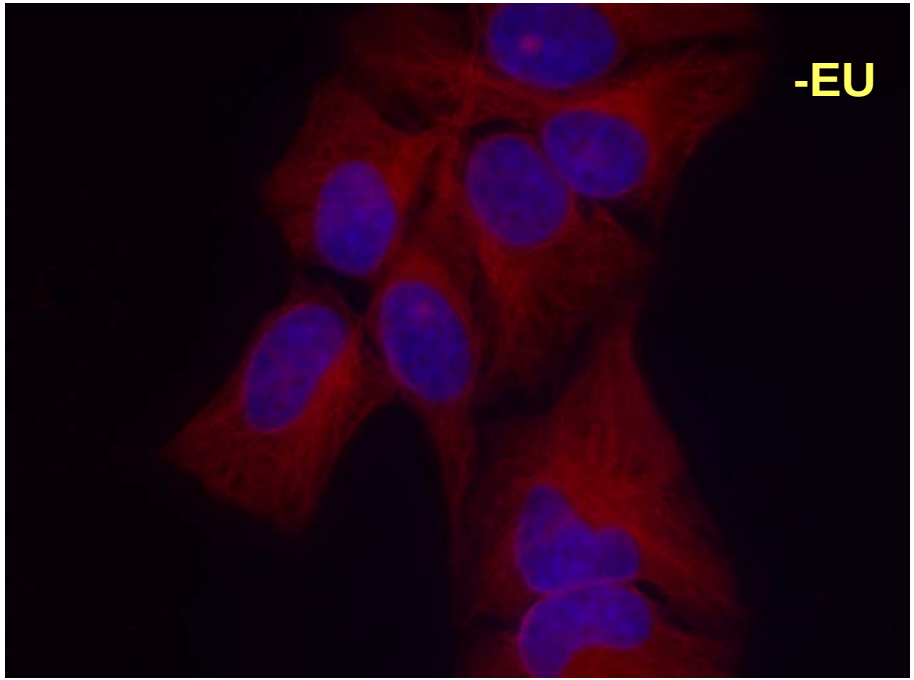
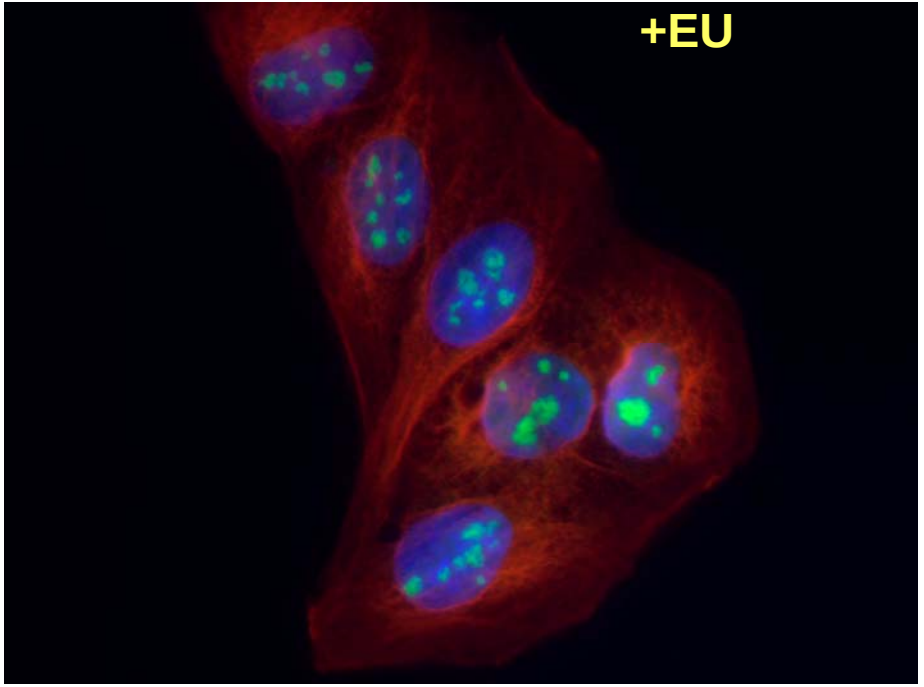
Nascent RNA Imaging with Click-iT® EU





HeLa cells +/- 1 mM EU for 1 hr followed by click reaction with Alexa Fluor® 488 azide (green), immuno-detection of tubulin with Alexa Fluor® 594 secondary (red), and Hoechst nuclear counterstain (blue).

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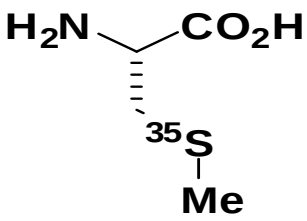
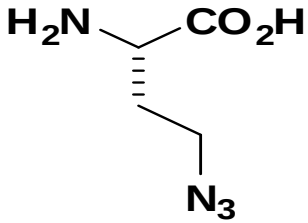
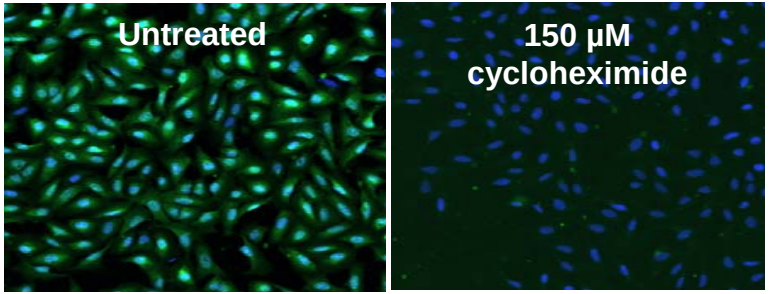
Click-iT® Nascent Protein Synthesis

35S-methionine

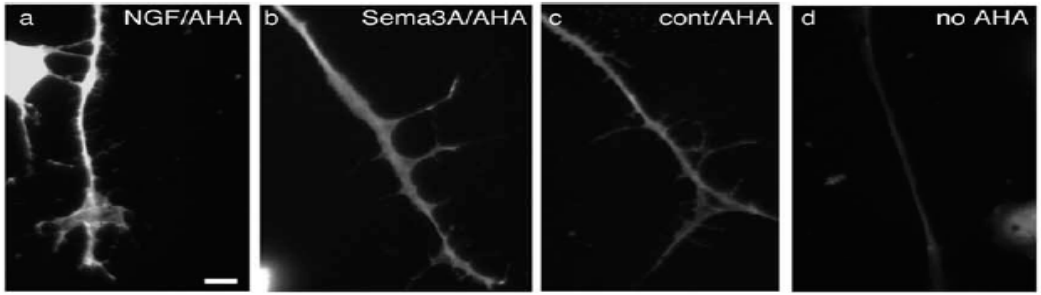




L-azido-homoalanine (AHA)



U-2 OS cells



The Journal of Neuroscience, January 21, 2009. 29(3): 638-652

- **Validated HCS kit that enables detection of pre-lethal effects of compounds on nascent protein synthesis**
- **Faster and safer alternative to radioactive methionine techniques**

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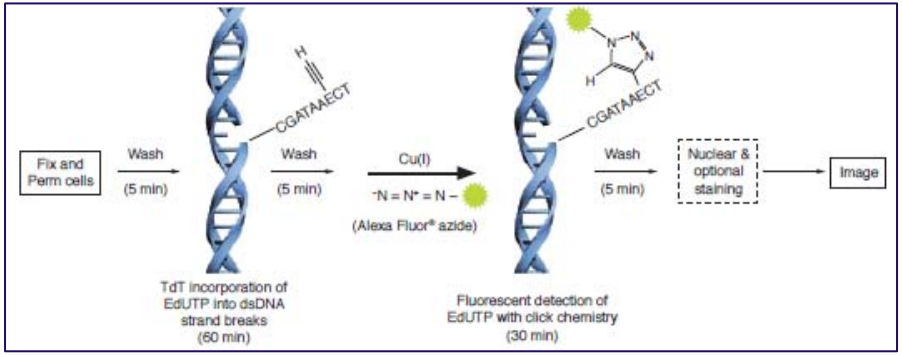
Click-iT® TUNEL Assay for DNA Damage

Stress/Ox DNA Nicking

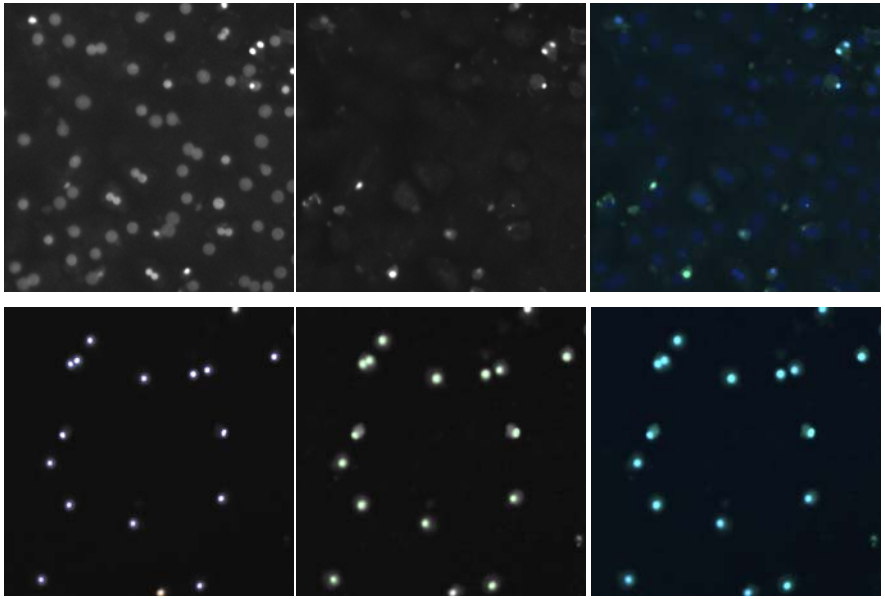


Primary human hepatocytes

Hoechst | Click-IT® TUNEL | Overlay

Control

10 µM Actinomycin D



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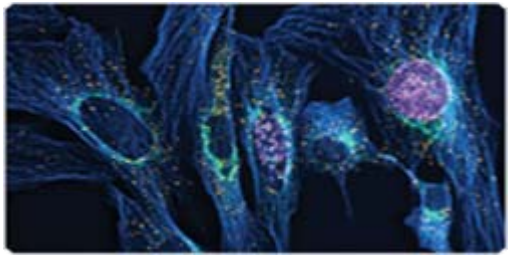
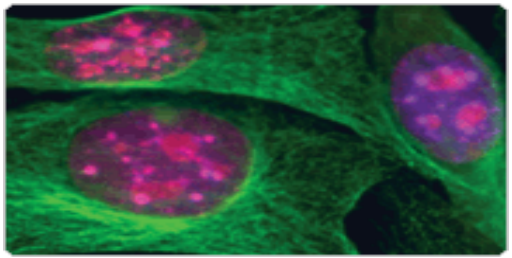
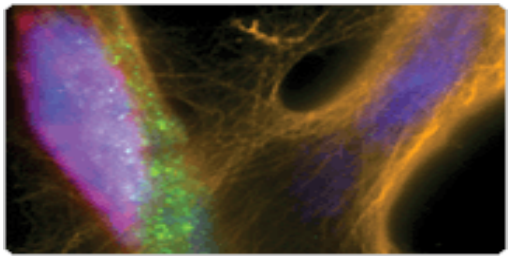
Click-iT® Detection Assays…



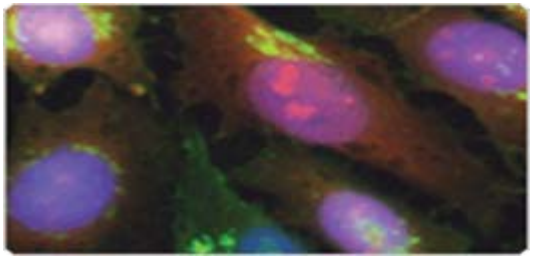
Nascent DNA synthesis/Cell Proliferation
Click-iT® EdU Cell Proliferation Assay



Nascent RNA Synthesis
Click-iT® RNA Assay



Detection of DNA Strand Breaks
Click-iT® TUNEL Assay for Apoptosis



Nascent Protein Synthesis
Click-iT® Detection of Protein Synthesis & Post Translational Modification

www.invitrogen.com/clickit

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Click-iT® Precursors, Labels and Applications

Azide/Alkyne Precursors

Nascent protein, DNA, RNA synthesis

Sugar PTM reagents
- Mannosylation
- Isoprenylation
- Fucosylation
- Fatty Acylation
- O-Linked Glycoproteins:
- Sialic Acid Modified Glycoproteins

TUNEL Assay

Azide/Alkyne Labels:

Alexa Fluor® Dyes

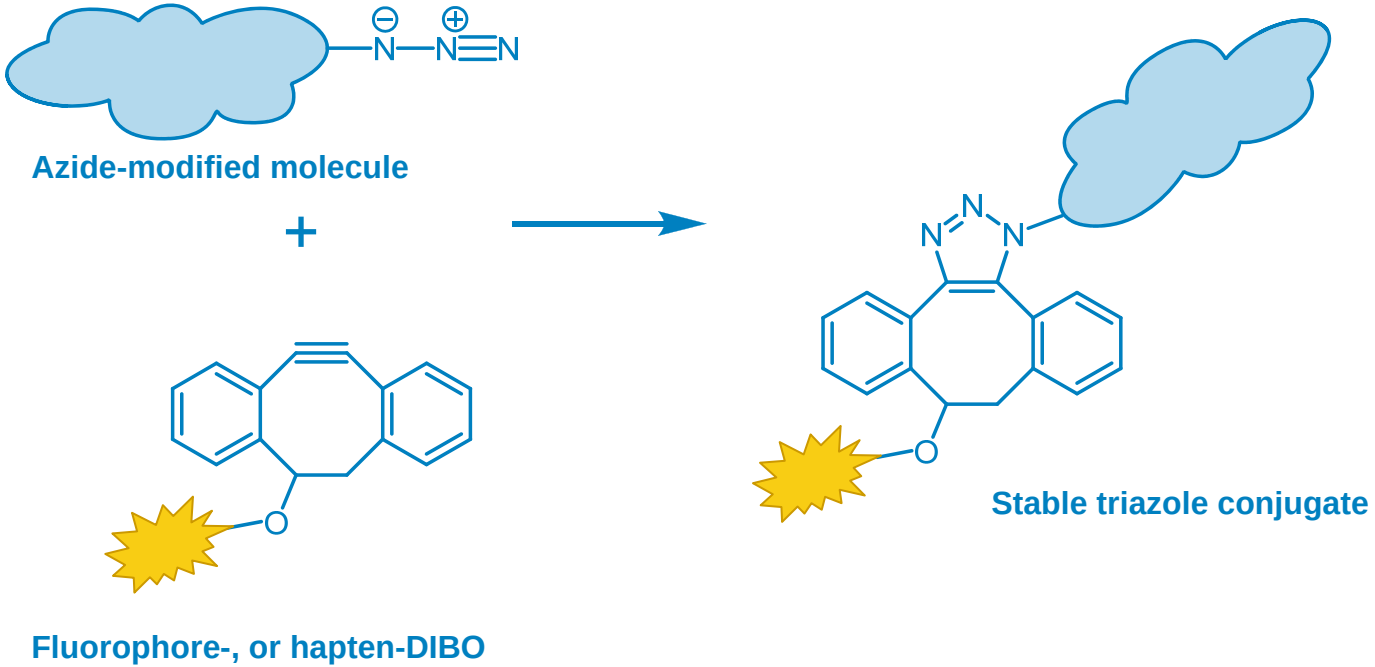
Avidin/Biotin

Oregon Green® 488

TAMRA

Etc..

Copper-free Click-iT® Reagents for Live Cells



DIBO Alkynes react with incorporated azide macromolecules

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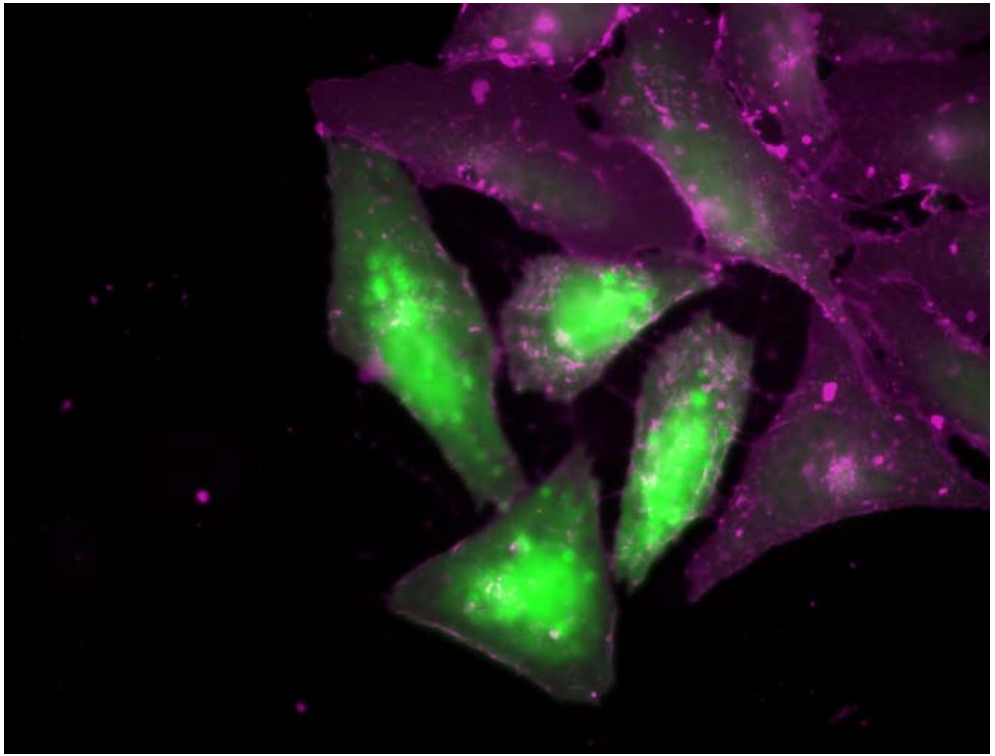
Copper-Free Click Mannosylation in GFP Cells



Label:
ManNAz Azido Mannose Label

Detect:
Alexa Fluor® 647 DIBO alkyne

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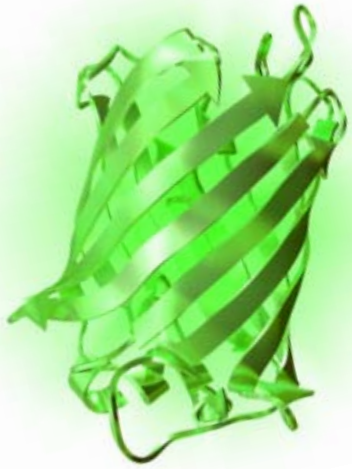
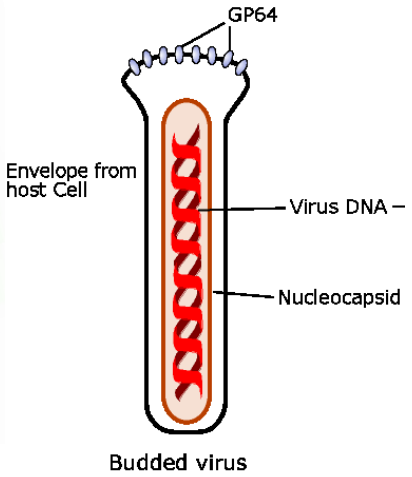
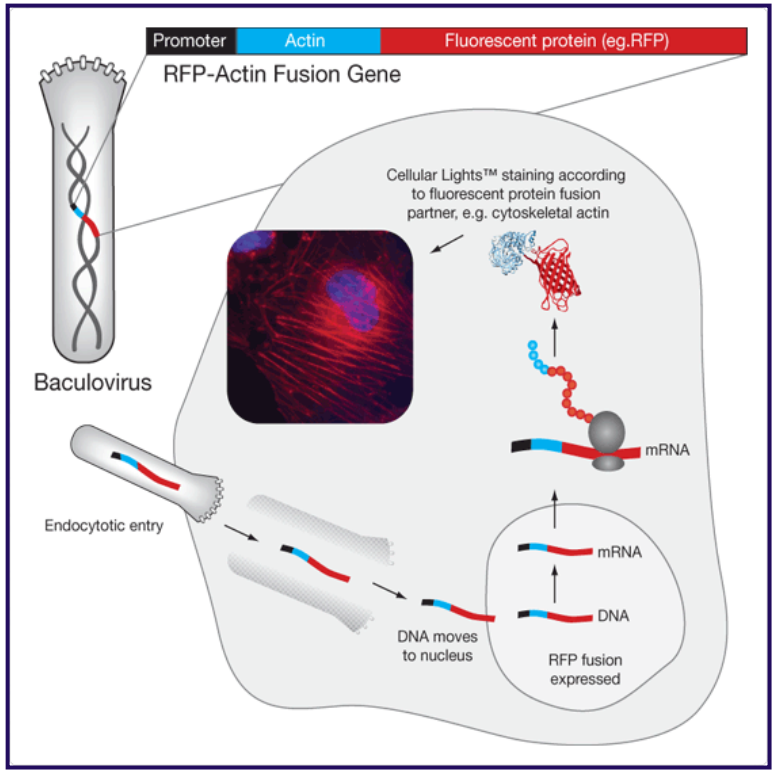
BacMam Gene Delivery





Targeted fluorescent proteins + BacMam



CellLight® Reagents

Premo™ Biosensors

BacMam Targets: Ion Channel, GPCR, Kinase etc

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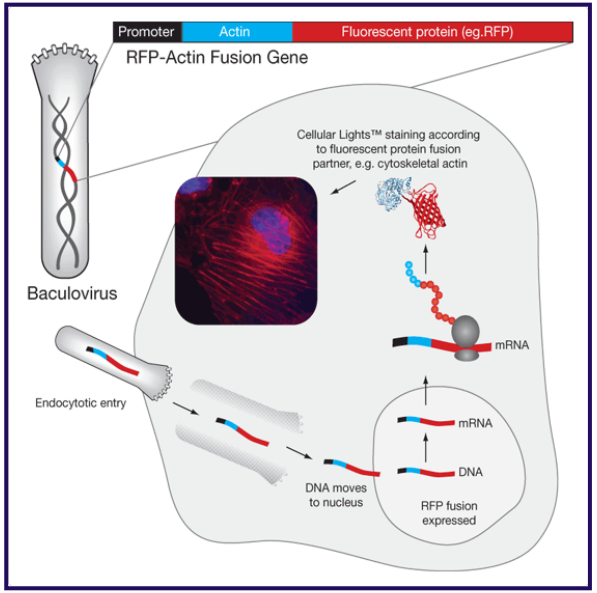
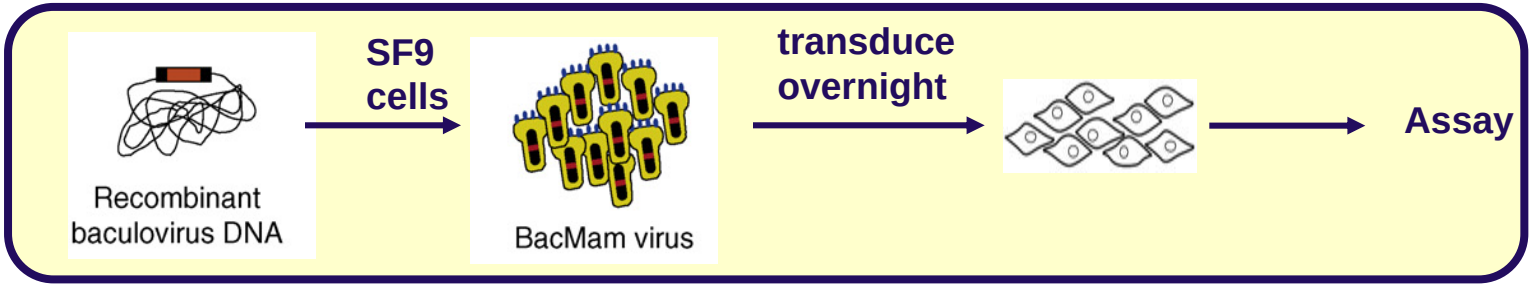
What is BacMam?



- **BacMam** is the use of modified **baculovirus** on mammalian cells in a way that is:
  - Convenient / easy-to-use
    - Just add to cells
  - Productive
    - High expression level
  - Safe
    - Non-toxic to cells and is BSL1
    - No footprint; non-replicating, non-integrating



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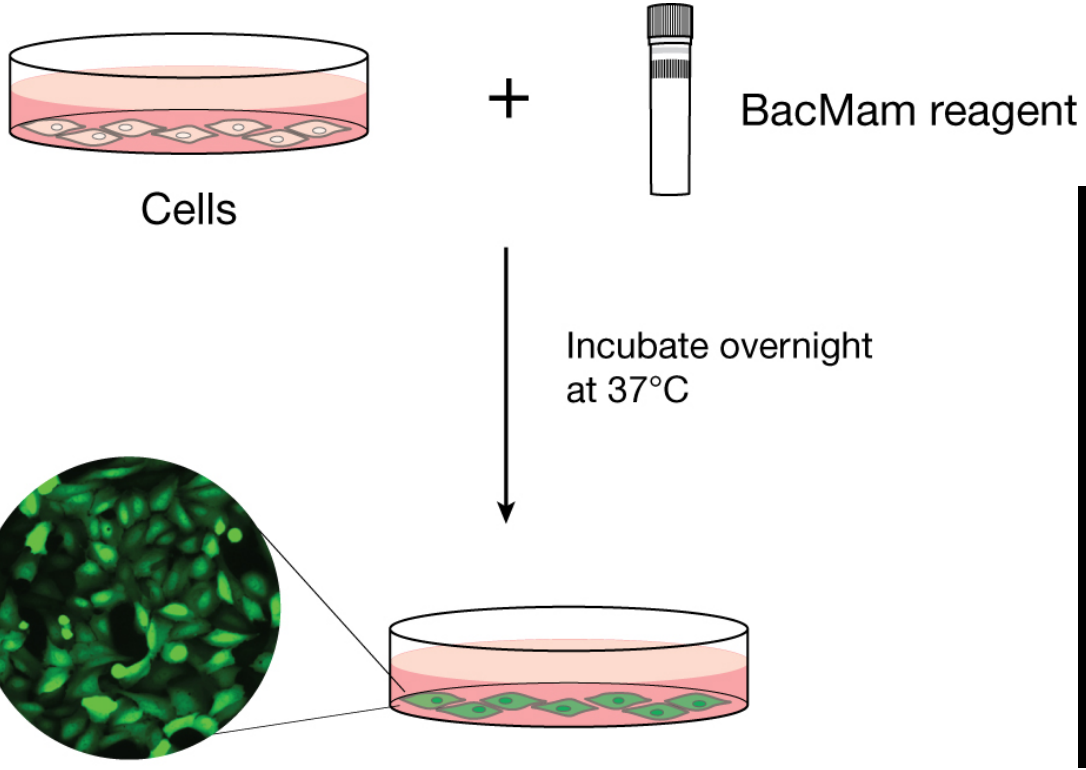
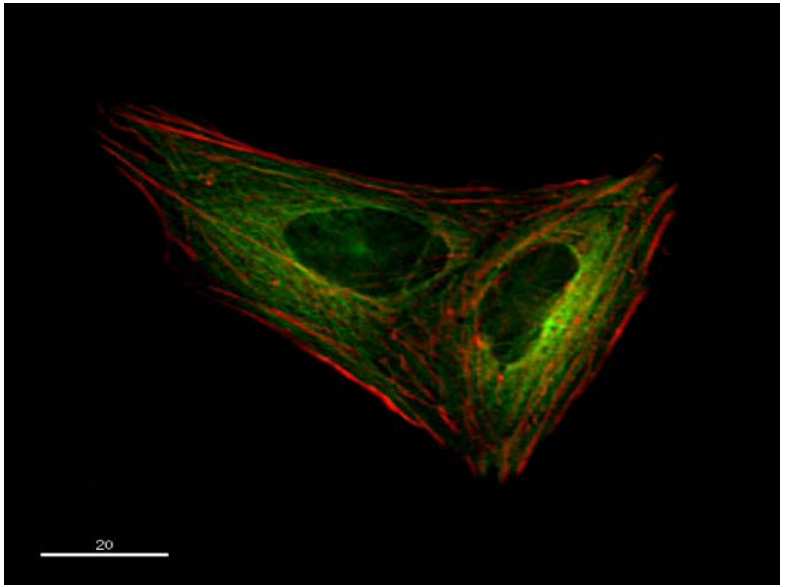
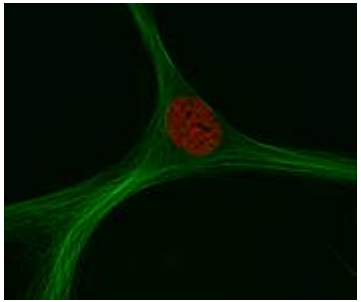
How Do I Use BacMam?



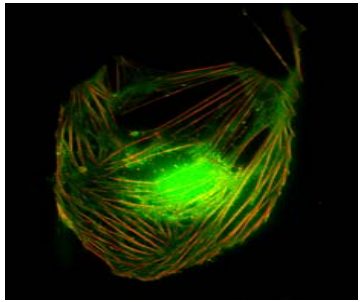


Actin RFP + Tubulin GFP

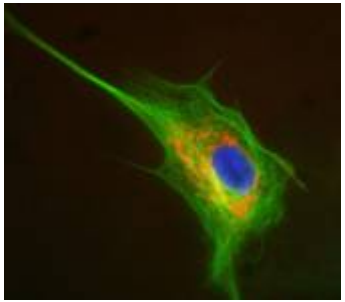
Primary Cells with No Toxicity



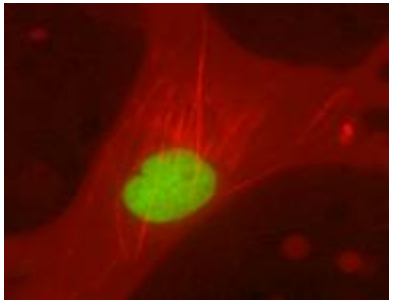
Human mesenchymal stem cell
H2B RFP MAP4 GFP



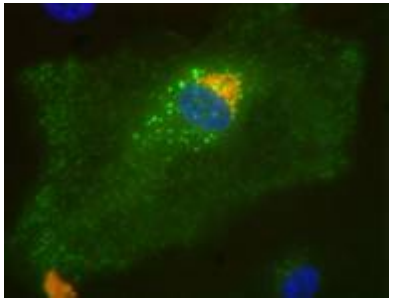
Adult Mouse Schwann Cell
GFP/Actin RFP



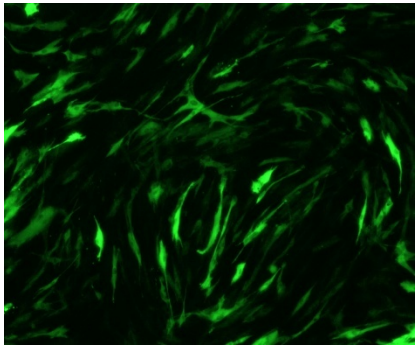
Human Aortic Smooth Muscle
ER RFP, Tubulin GFP
DAPI



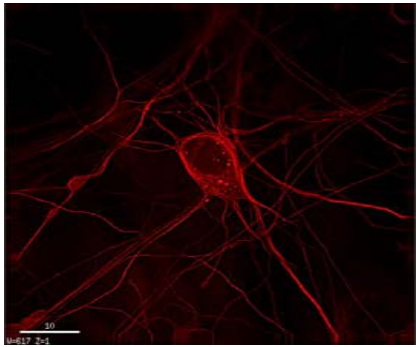
Human airway epithelial smooth muscle
Actin-RFP, Histone 2B-GFP



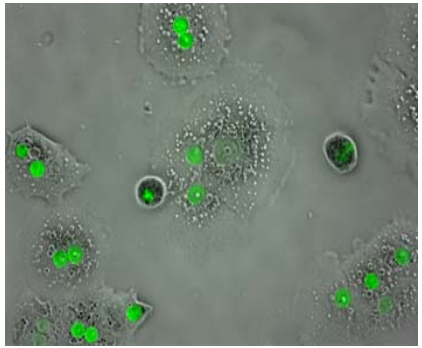
Human umbilical vein endothelial cell
Endosome-GFP, Golgi-OFP



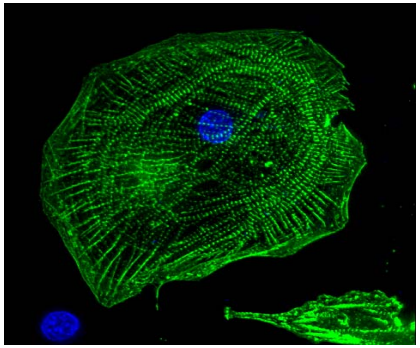
Adipose Derived Stem Cells
GFP Transduction Control



E18 Rat Hippocampal Neurons
RFP Tubulin

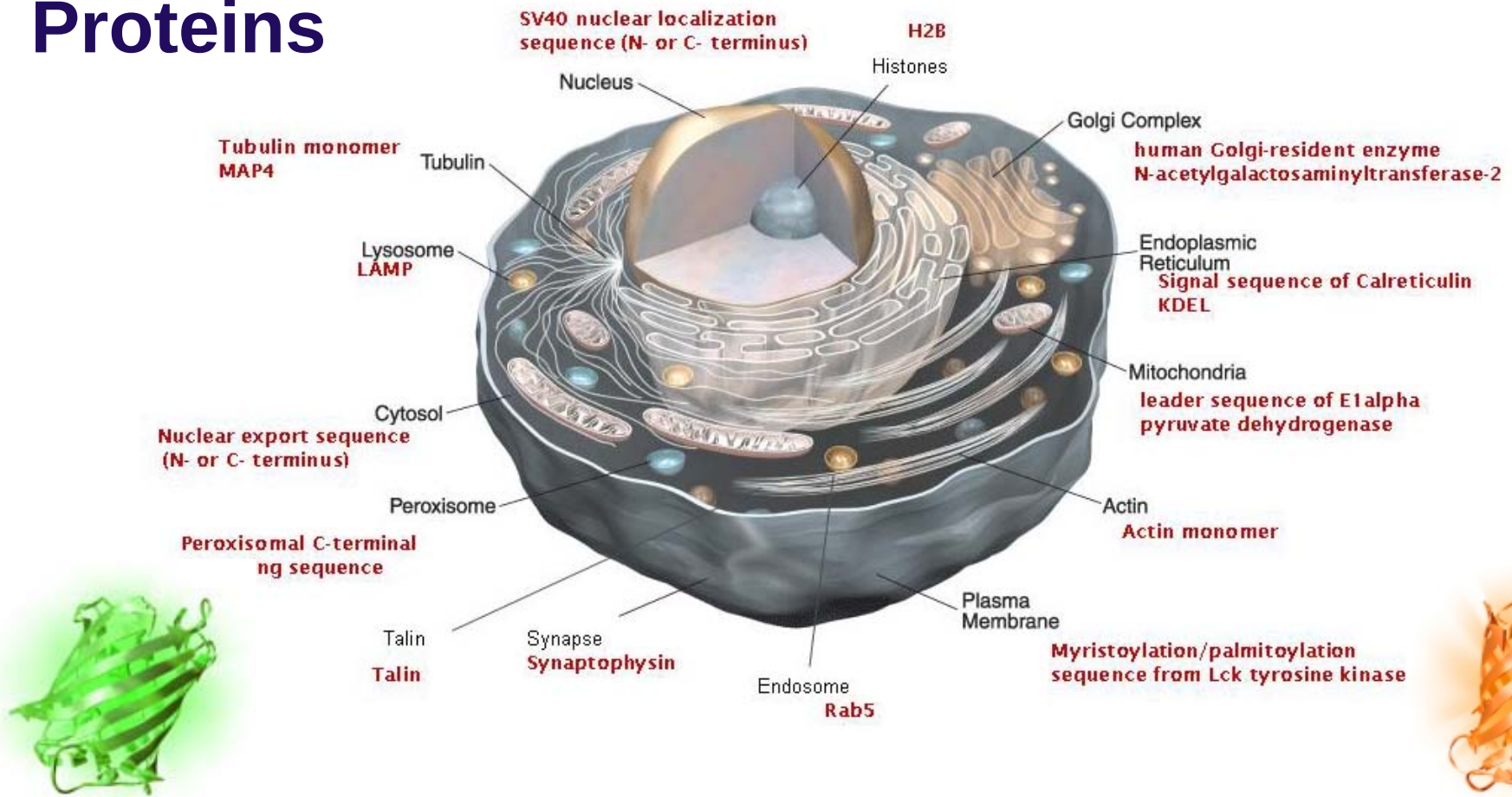


Human Hepatocytes
GFP H2B

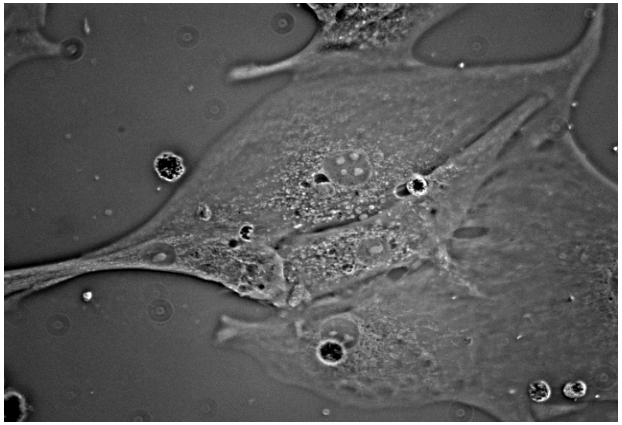


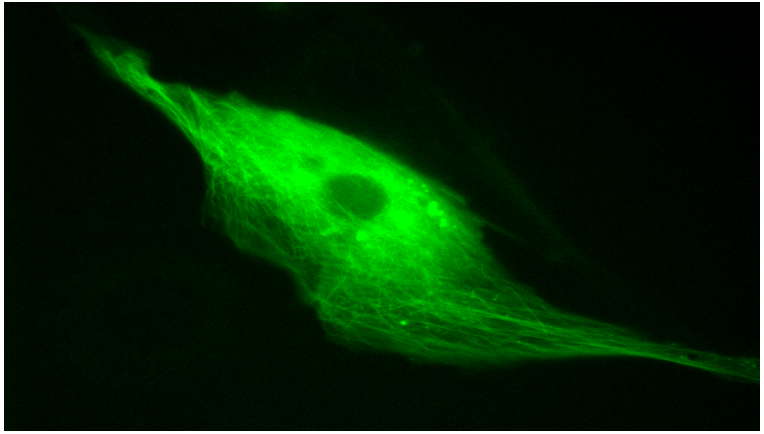
Mouse Cardiomyocyte
GFP Actin, Hoerschst

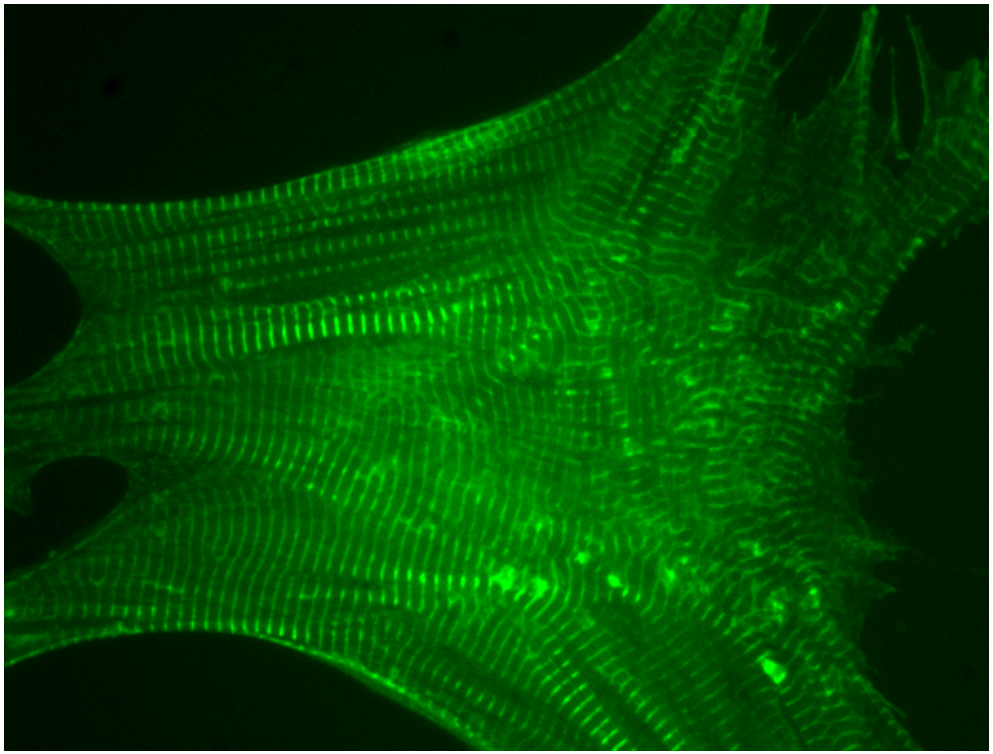
BacMam CellLights® Targeted Fluorescent Proteins



Mouse Cardiomyocytes







Actin GFP

Tubulin GFP

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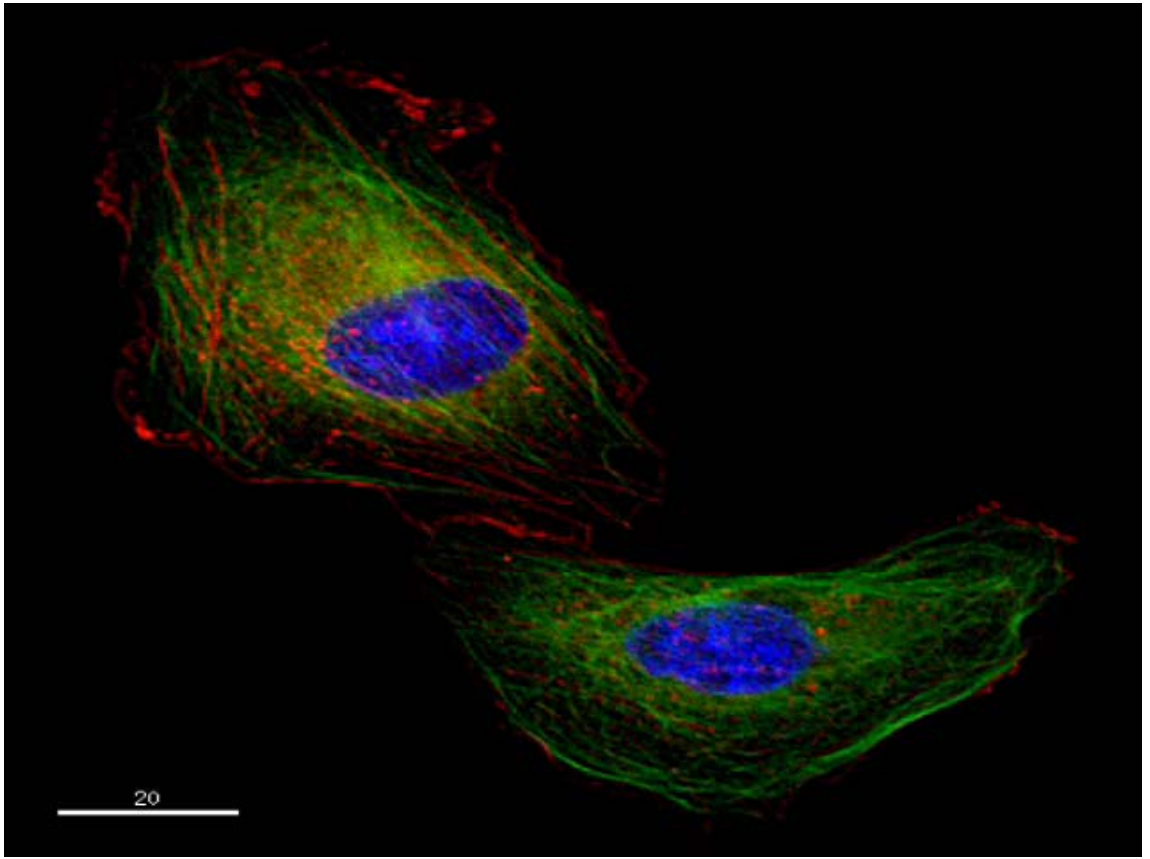
Actin Stress Fiber Disruption

Cytochalasin D

Dissolves actin filaments
Tubulin network intact

Tubulin GFP

Actin RFP



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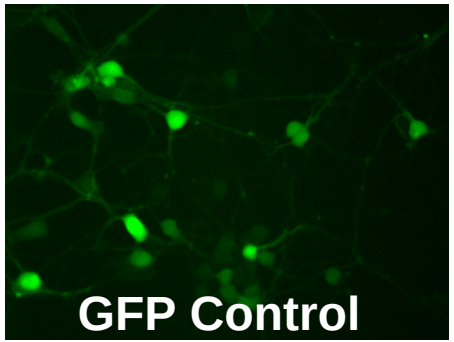
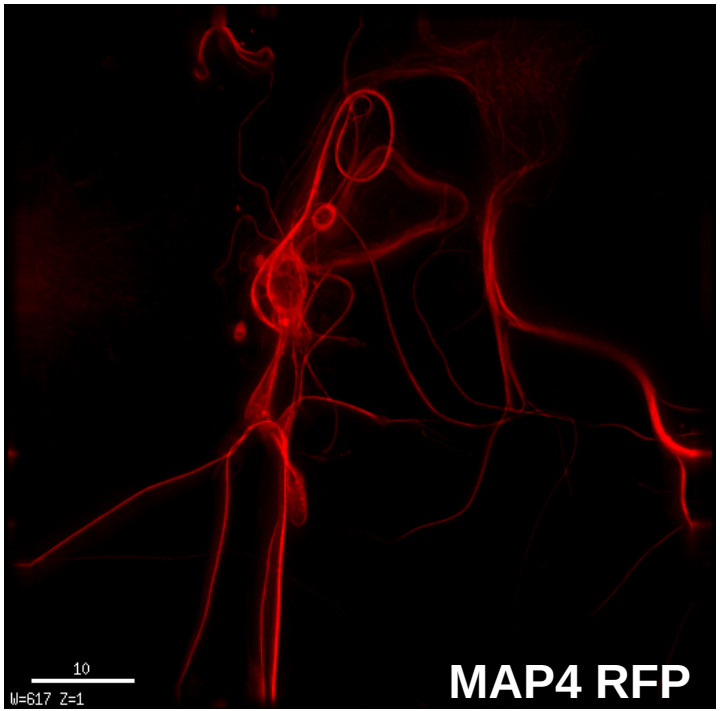
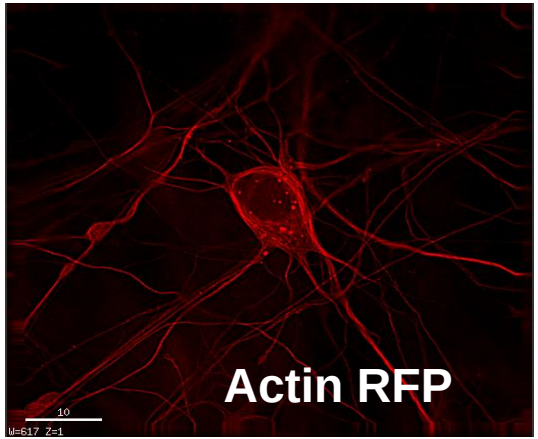
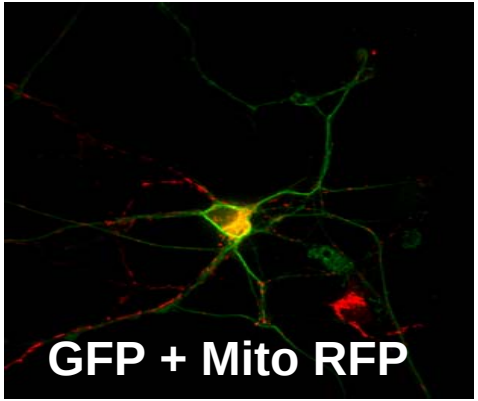
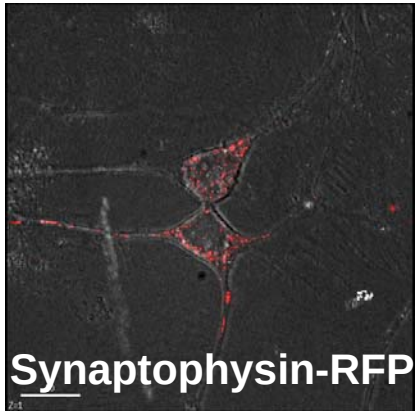
Hippocampal Neurons











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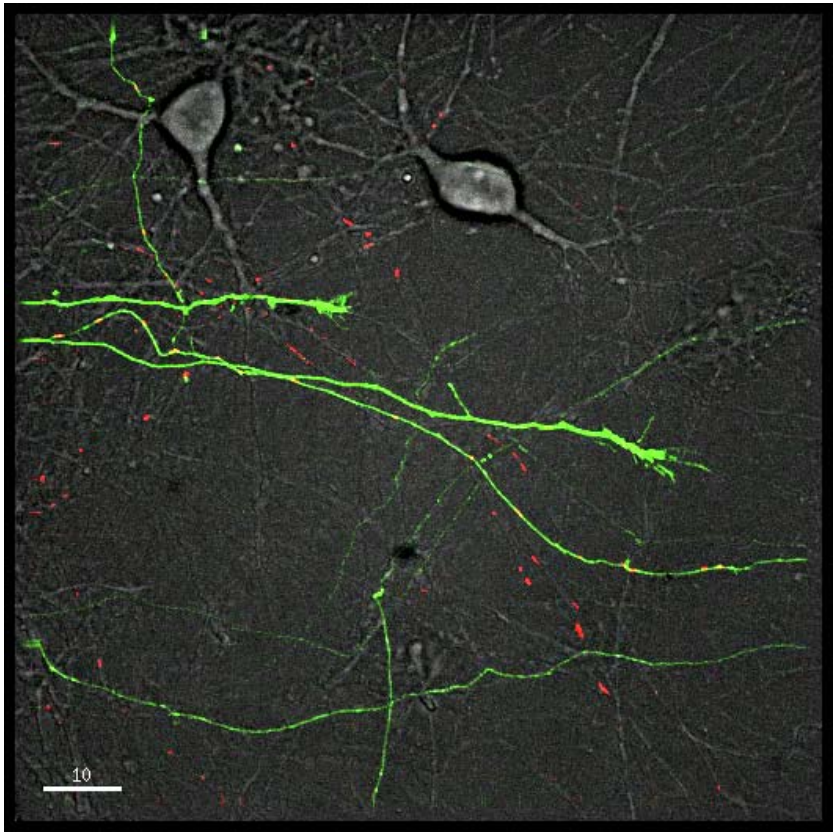
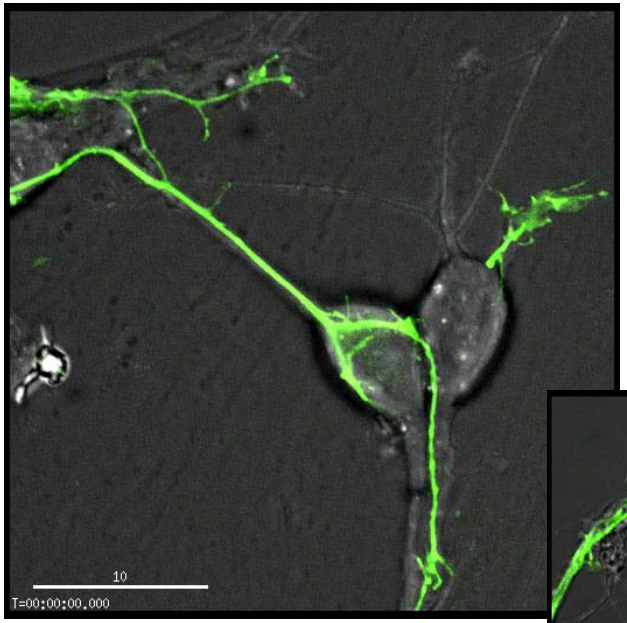
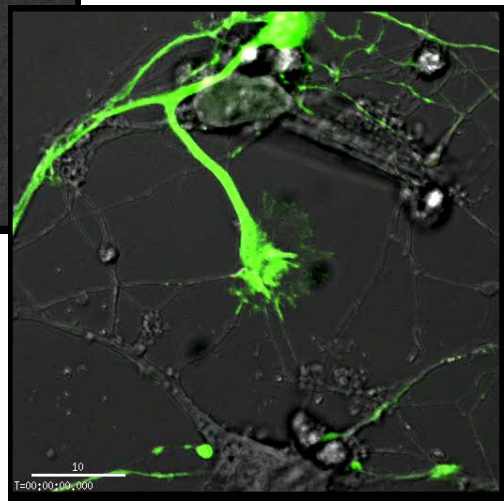
Growth Cone Dynamics and Mito Traffic

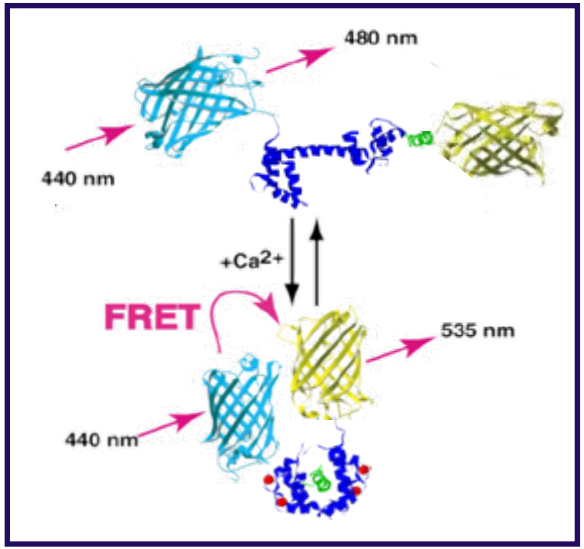


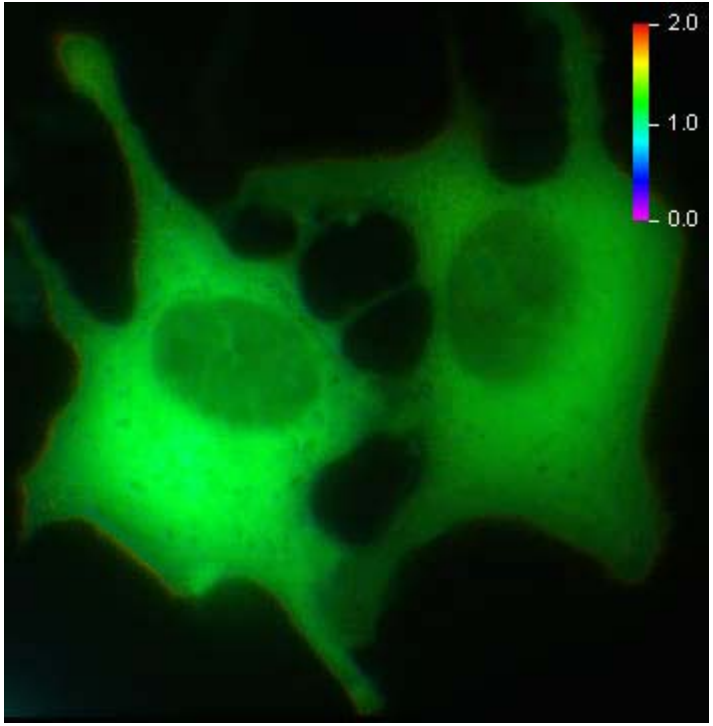




GFP Control
Mito RFP

Premo™ Cameleon Calcium Sensor





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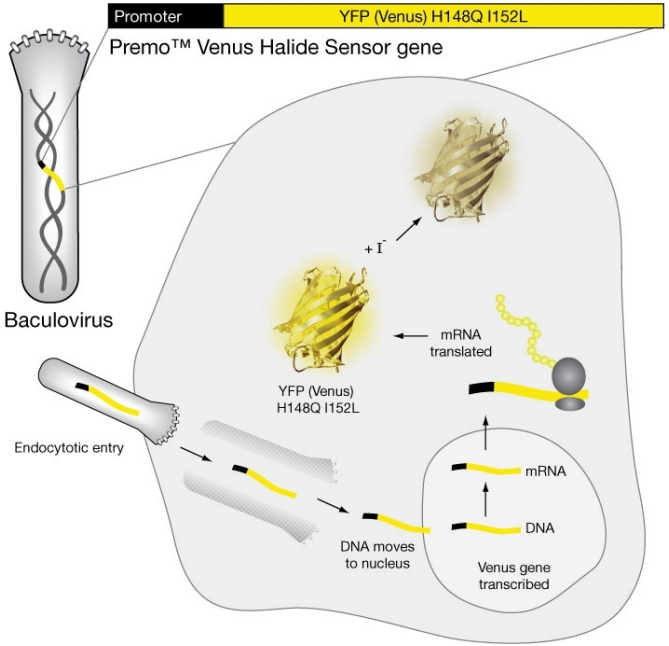
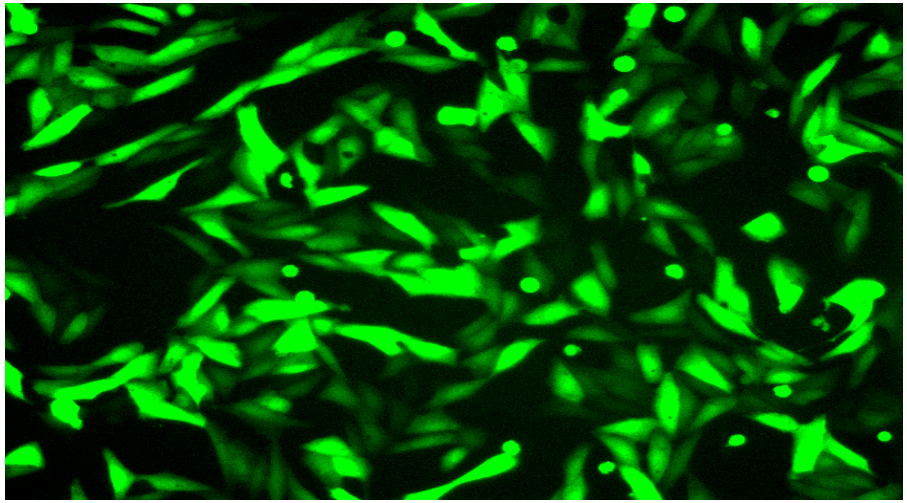
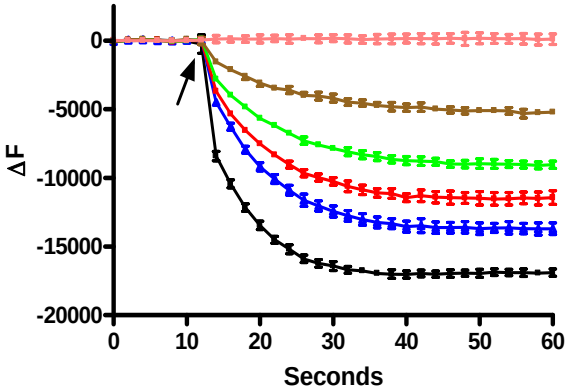
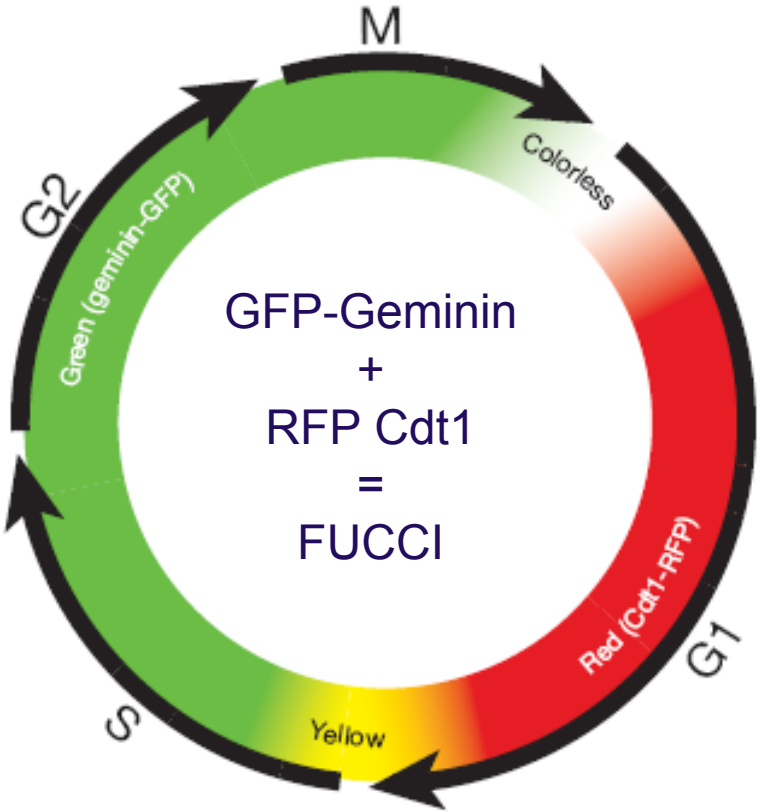
Premo™ Halide Sensor

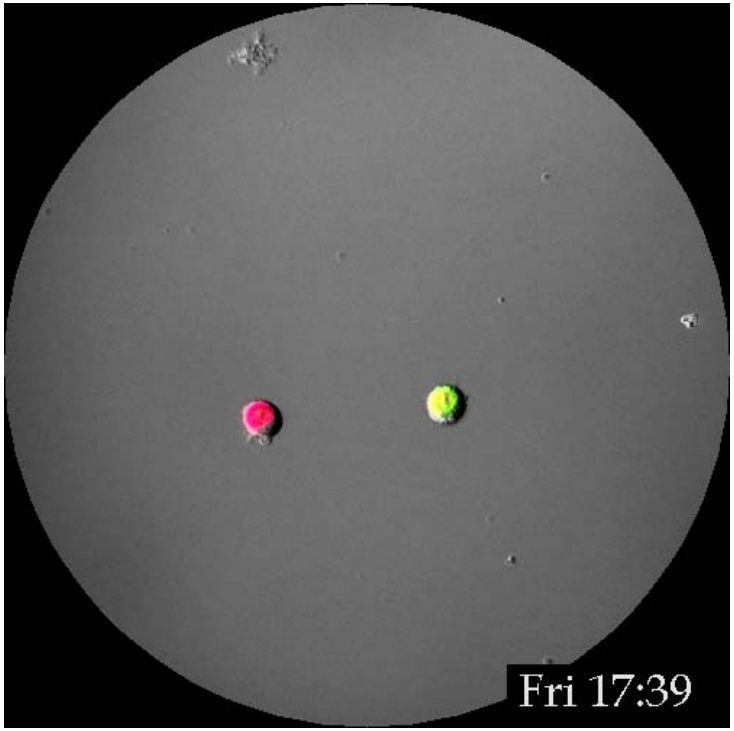






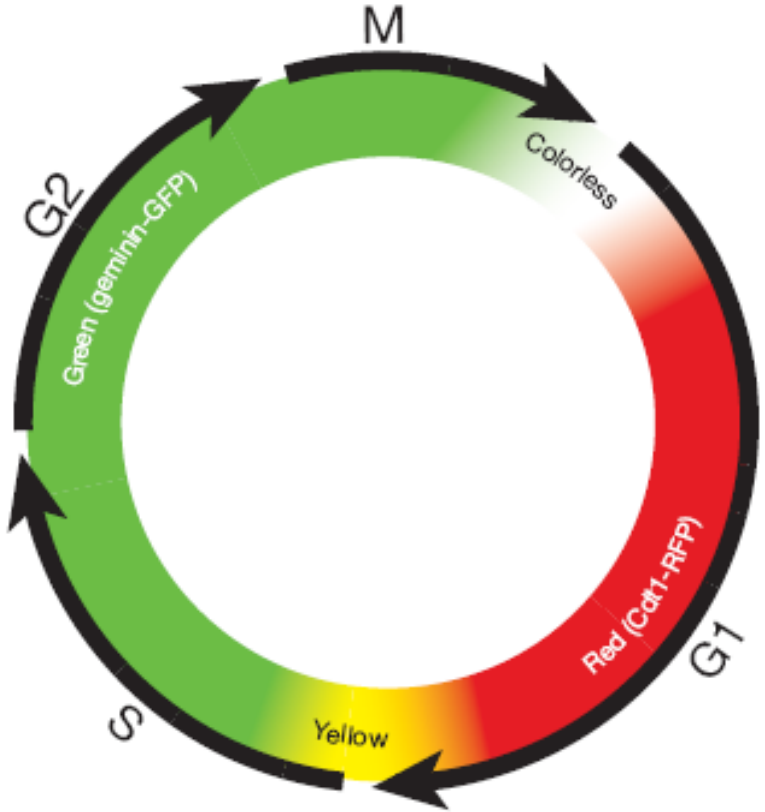
FUCCI Cell Cycle Sensor





Atsushi Miyawaki Lab, Riken Institute

BacMam Premo™ FUCCI Cell Cycle Sensor



Visualizing spatial and temporal aspects of cell cycle progression with fluorescent proteins via BacMam gene delivery technology

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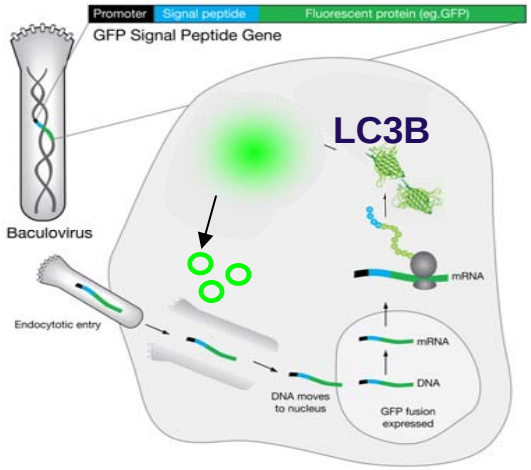
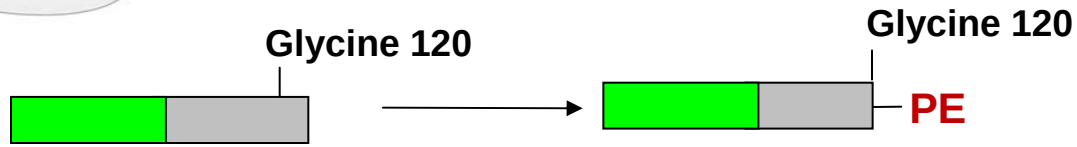
Premo™ LC3B Autophagy Sensor



- Fusion between LC3B and GFP/RFP
- Expressed by CMV-E1 on baculovirus chromosome
- Delivery via BacMam 2.0 technology
- Transient expression in wide range of mammalian cells
- Live-cell indicator of autophagy

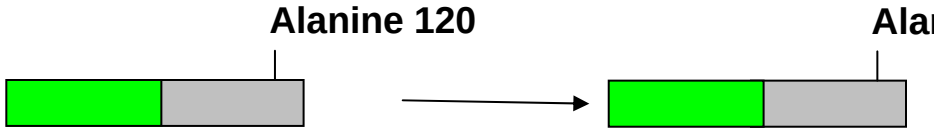
LC3B wt



Autophagosome formation

Pro autophagic stimulus

LC3B mutant (control)



No Autophagosome formation

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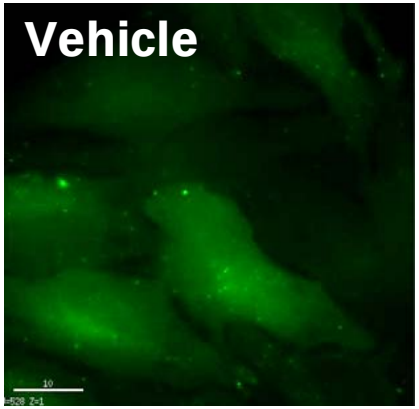
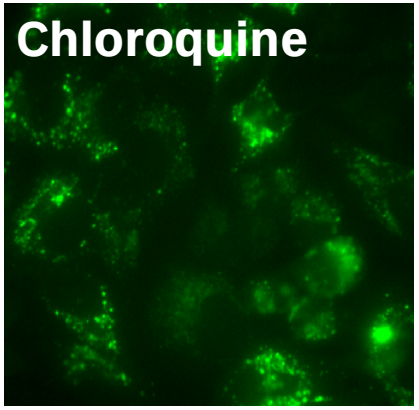
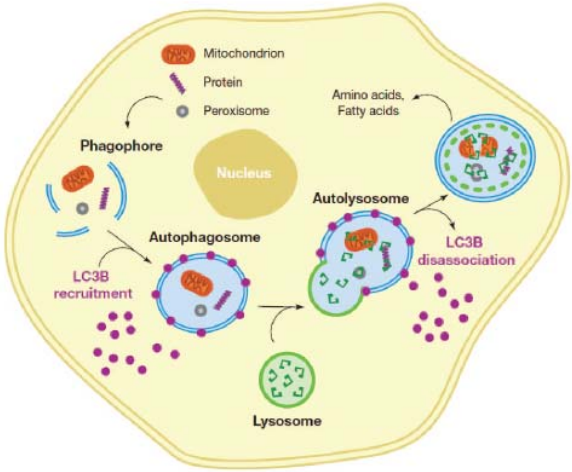
Premo™ LC3B Autophagy Sensor

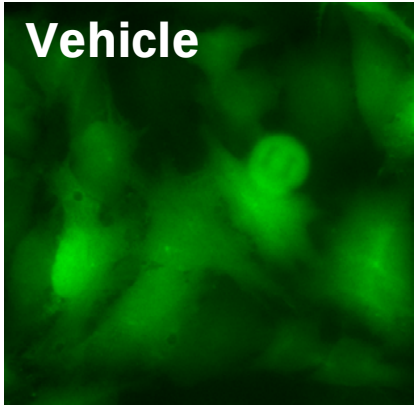
LC3B

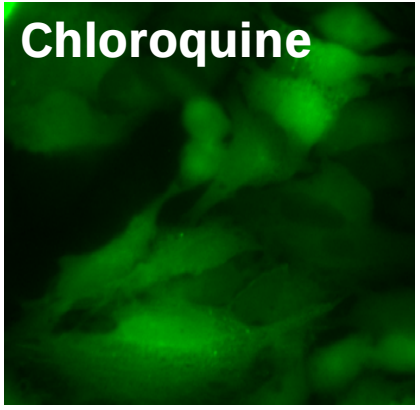


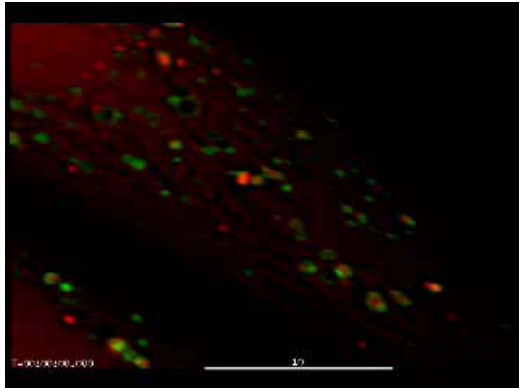




Mutant







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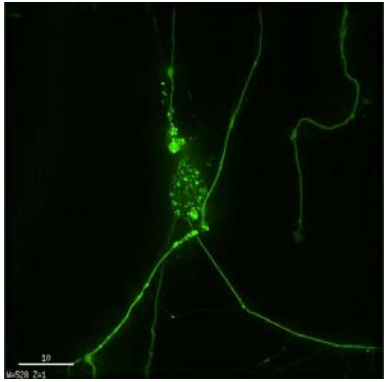
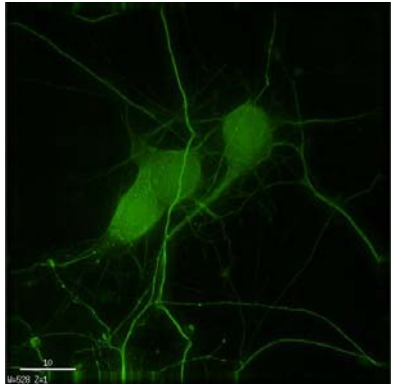
Autophagy in Primary Animal and Human Cells

Rat Hippocampal

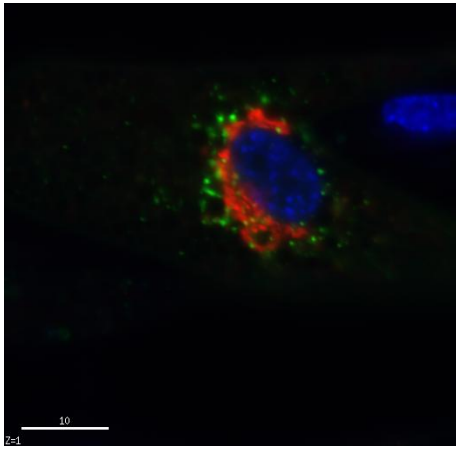
LC3B G120A

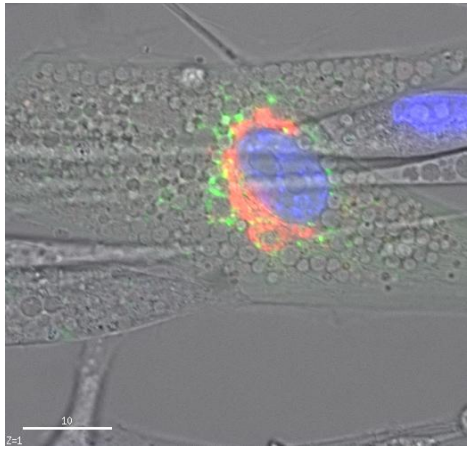


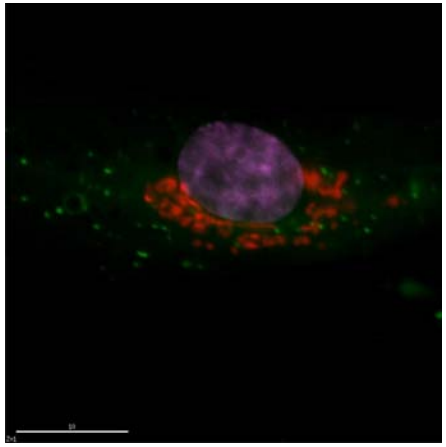
WT LC3B



Human Aortic Smooth Muscle Cells







BacMam LC3B-GFP + CellLight® Golgi-RFP

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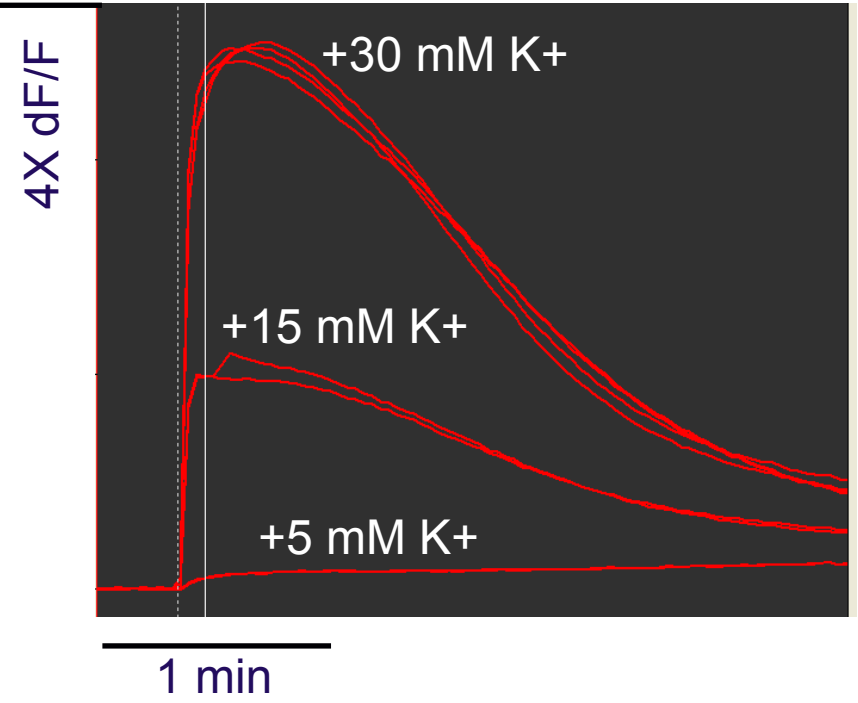
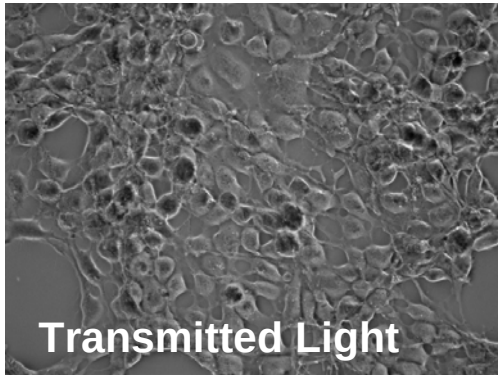
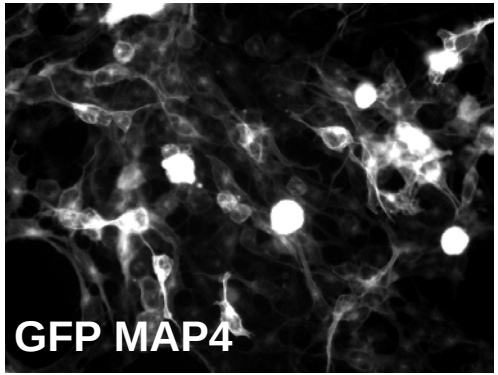
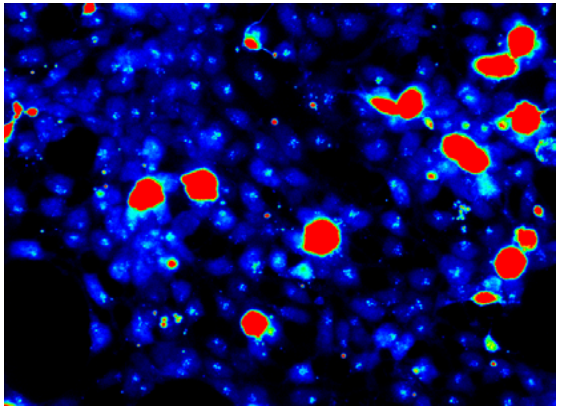
Rhod-3 Calcium Imaging with CaV2.1

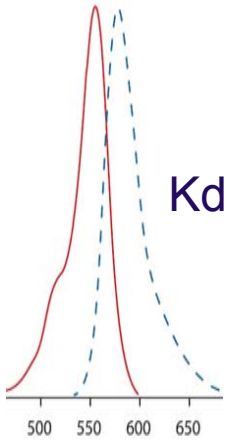


BacMam CaV2.1 α + β +α2δ









Kd 570 nM

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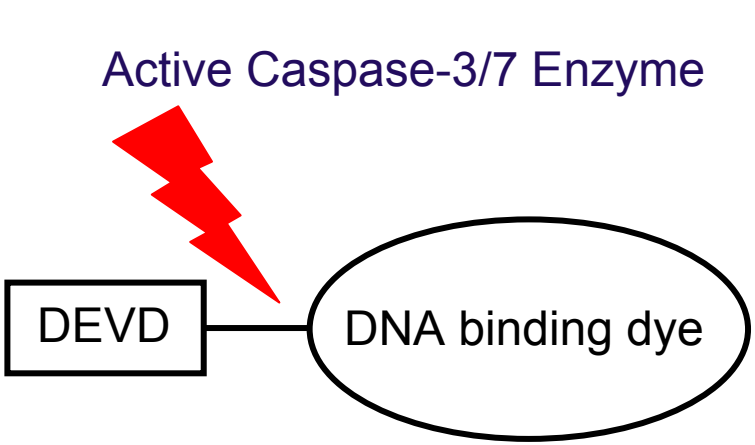
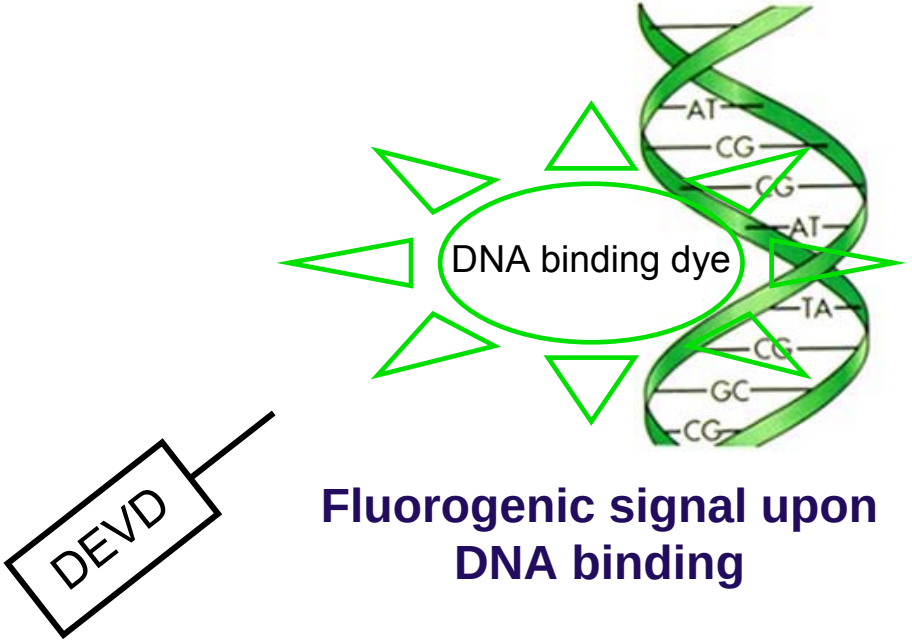
CellEvent™ Caspase 3/7 Green Apoptosis Sensor

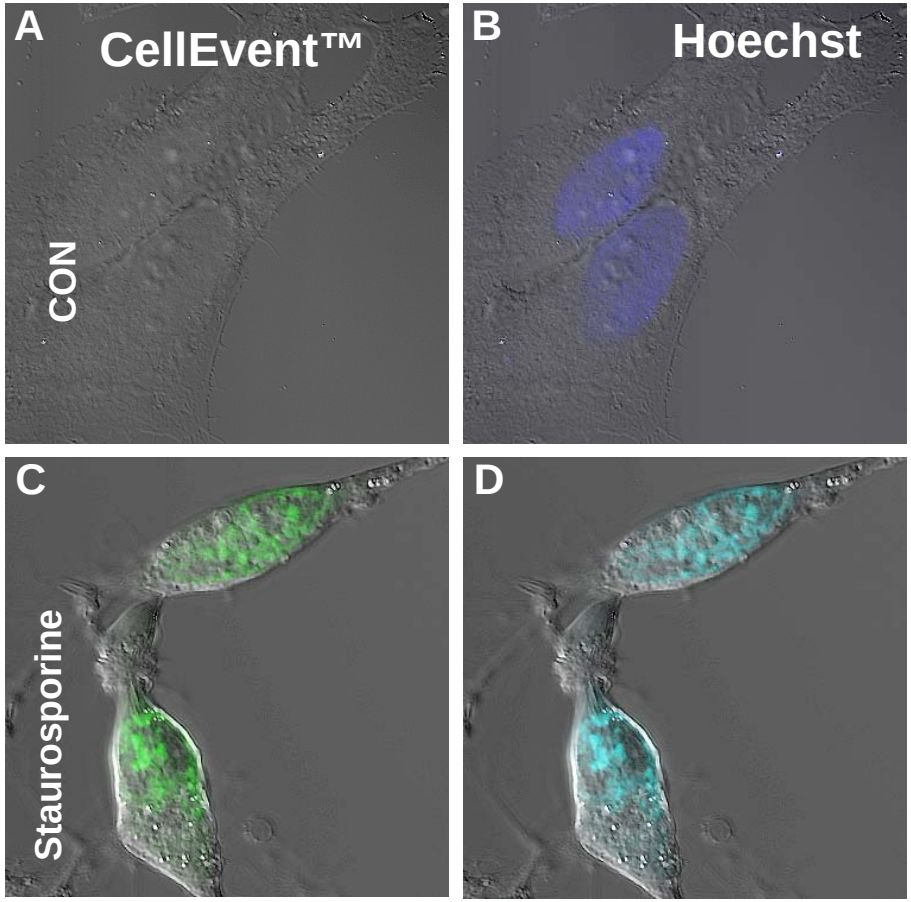


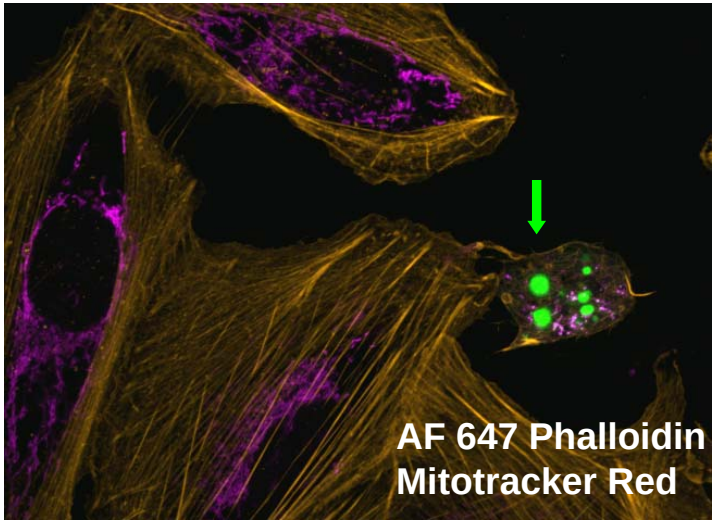
Non-fluorescent
No DNA binding

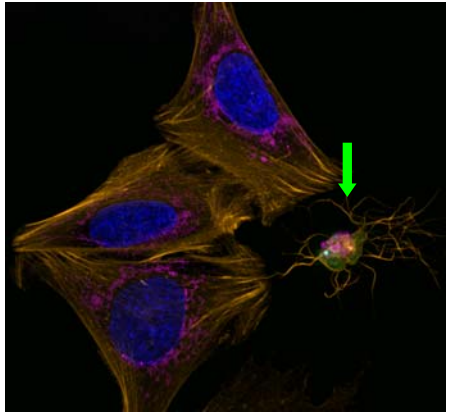


Fluorogenic signal upon DNA binding

CellEvent™ Caspase 3/7 Imaging







AF 647 Phalloidin
Mitotracker Red
Hoerchst

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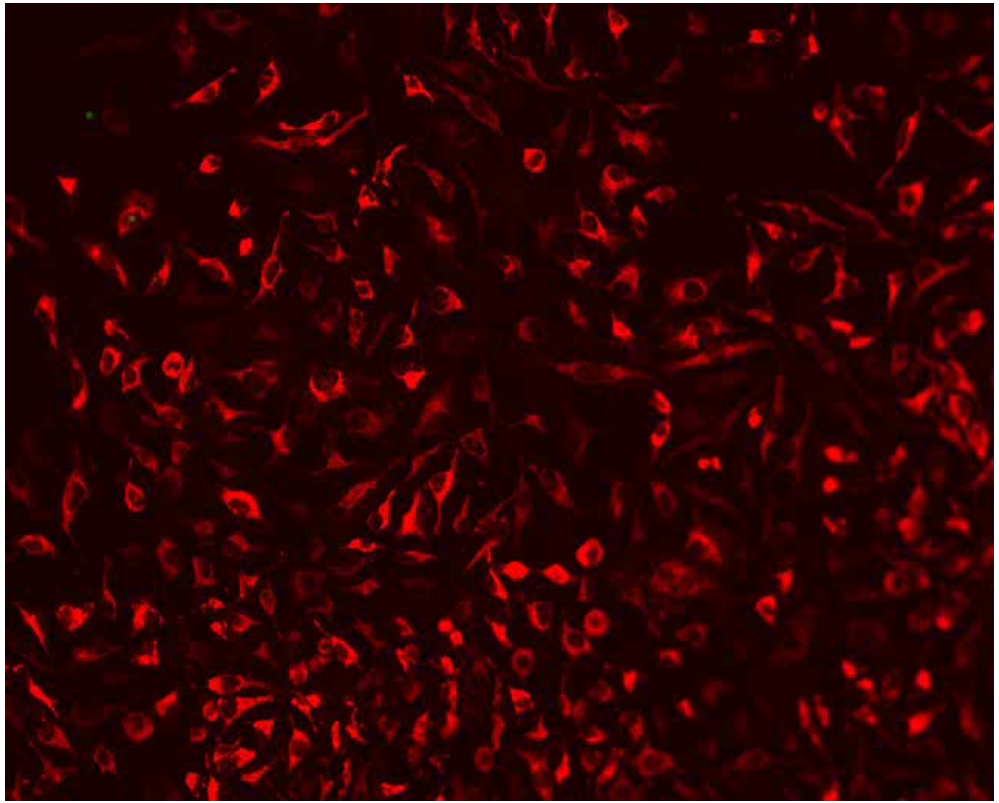
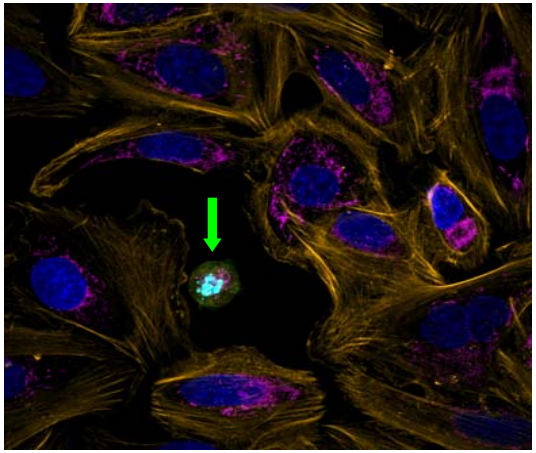
CellEvent™ Caspase 3/7 Time Lapse in HCS

ImageXpress Micro®



Red: TMRM Mitochondrial Vm Dye
Fades with apoptosis

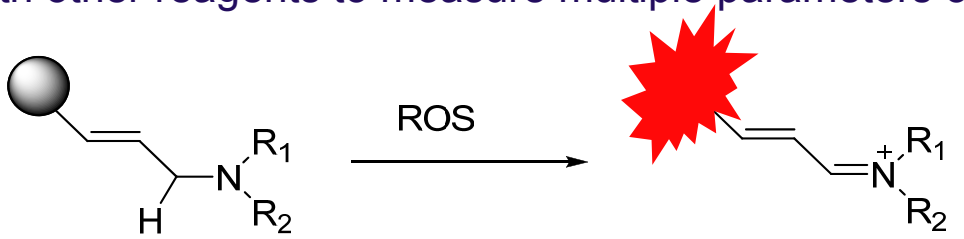
Green: CellEvent™ Caspase 3/7
Fluorogenic with apoptosis



Staurosporine Time Lapse

CellROX™ Deep Red ROS Sensor

1. Dihydrodichlorofluoresceins are traditionally used for ROS measurements.They have to be added to serum free media and have higher backgrounds
2. CellROX™ Deep Red is a fluorogenic probe to measure oxidative stress in live cells.
3. Can be added to complete growth media.
4. CellROX™ Deep Red Reagent gives good S/N ratios and can be used with traditional fluorescence microscopy, high content screening, flow cytometry and flourescence plate reader assays.
5. CellROX™ Deep Red Reagent can be used in GFP-expressing cells and can also be multiplexed with other reagents to measure multiple parameters of cell health in the same cell.



Reduced (Nonfluorescence)

Oxidized (Fluorescence)

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CellROX™ Deep Red Staining

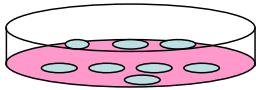
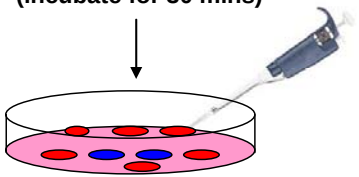


Plate cells
(suspension cells, if using
flow cytometry, are grown in flasks)

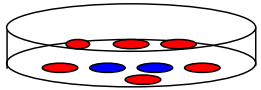
Drug treatment

Add CellROX™ Deep Red reagent & Hoechst 33342

(incubate for 30 mins)

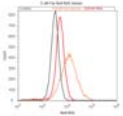


Wash cells 3X in PBS
(Suspension cells are washed by centrifugation)

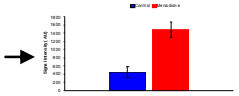


Analyze

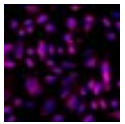
Flow Cytometry

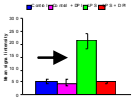


Fluorescence
Plate Reader

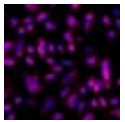


High content
screening





Traditional
Fluorescence
Microscopy



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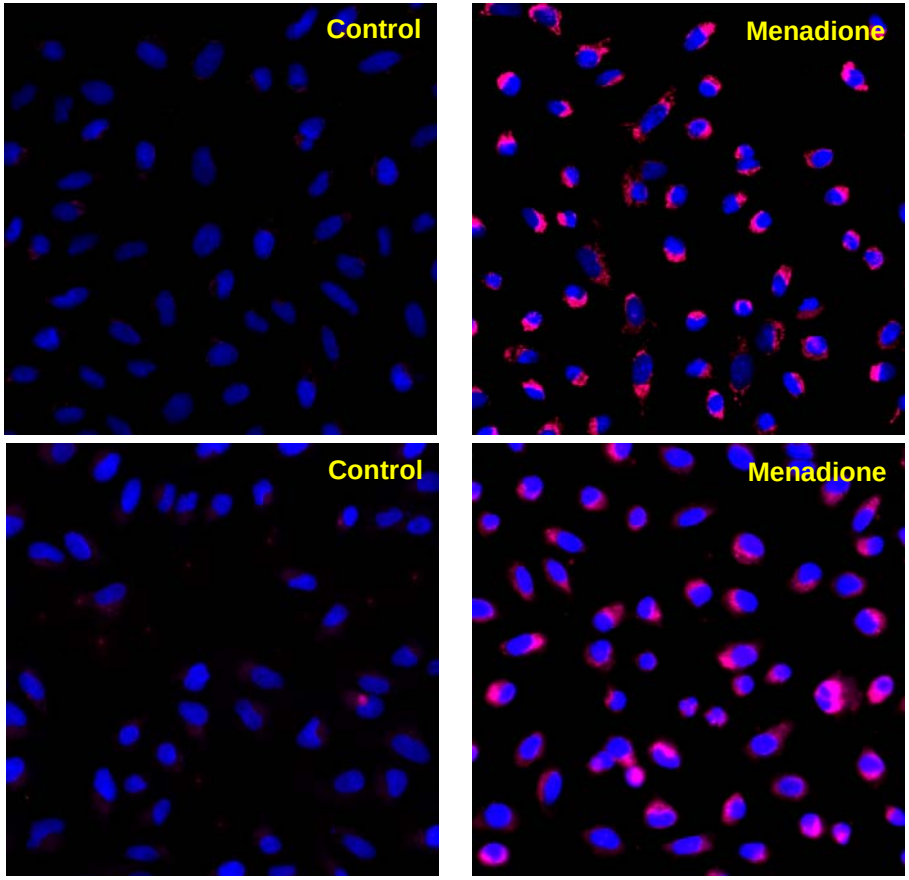
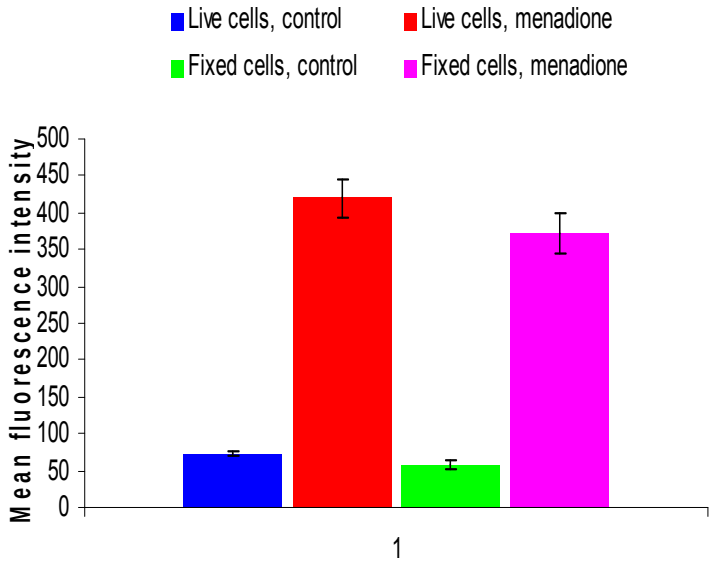
CellROX™ Deep Red ROS Sensor

Live cells

Fixed cells





U-2 OS Cells

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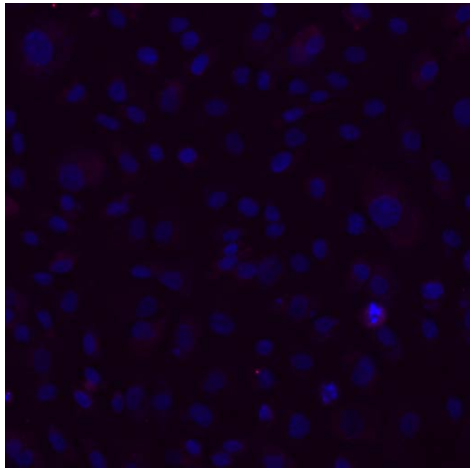
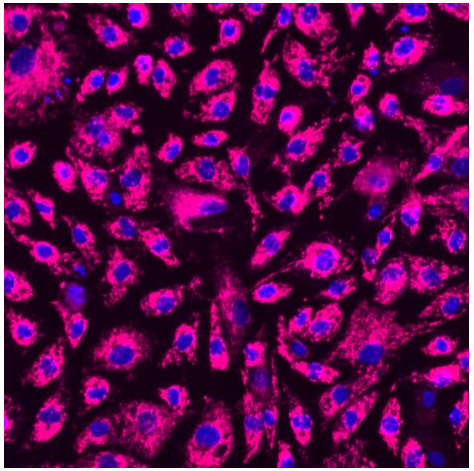
Reactive Oxygen Imaging

Control

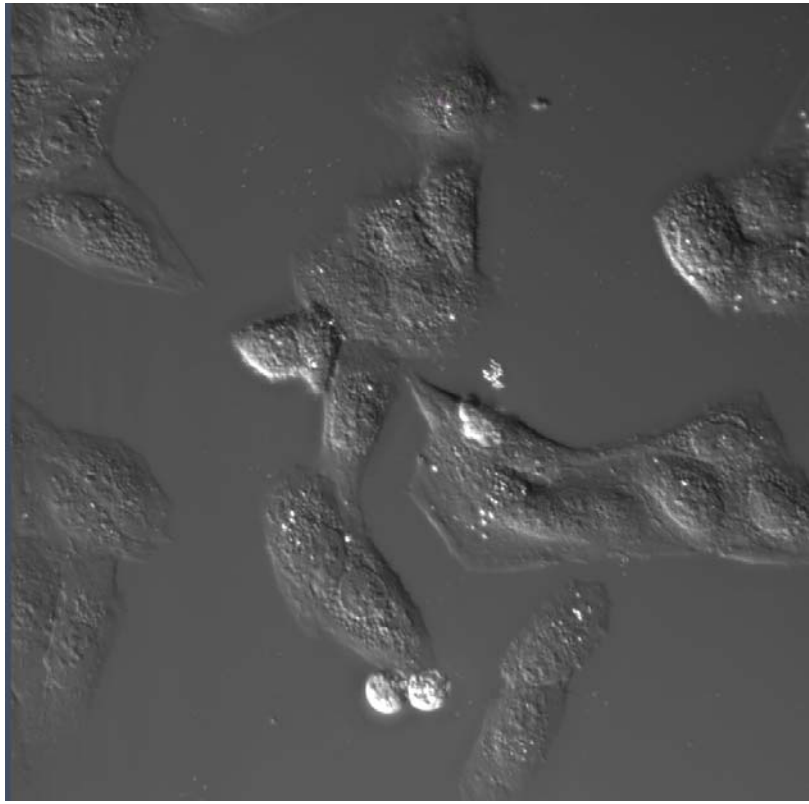


100 µM Menadione



Hoechst/CellROX™ Deep Red reagent

BPAE Cells



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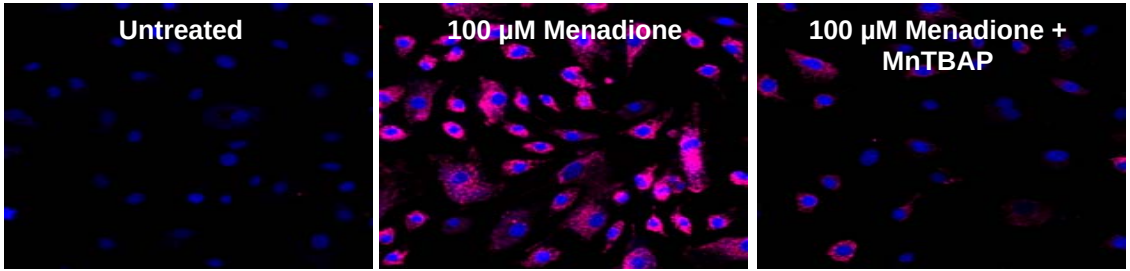
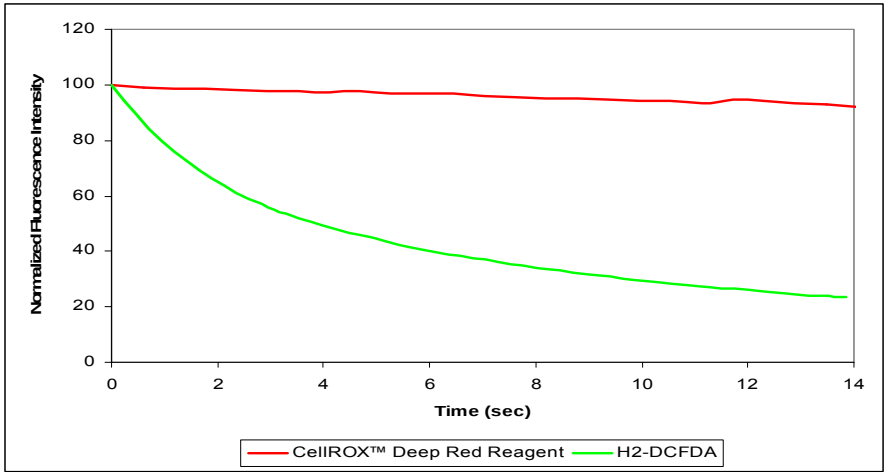
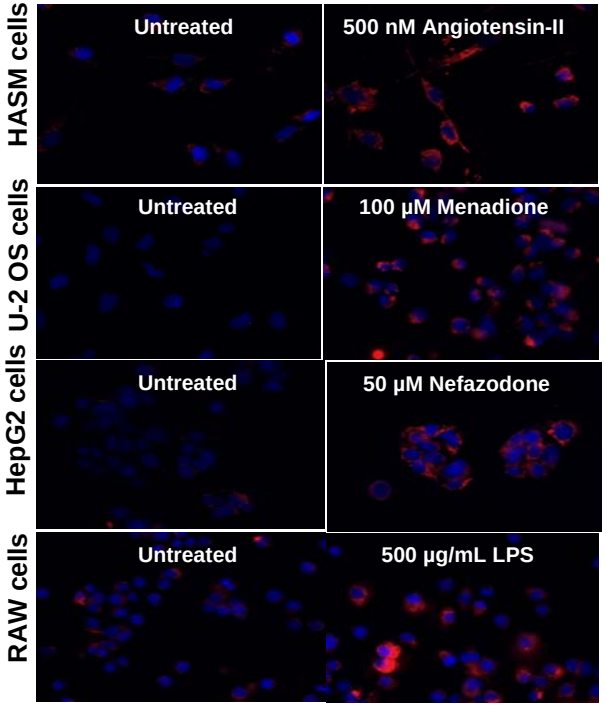
CellROX™ Deep Red reagent for detection of oxidative stress



Hoechst and CellROX™ Deep Red reagent added to live bovine pulmonary artery endothelial cells in complete media





Reduced fluoresceins, traditionally used for cellular ROS measurements, must be added to serum-free media and suffer from high background, photo-oxidation, photo-instability, etc.

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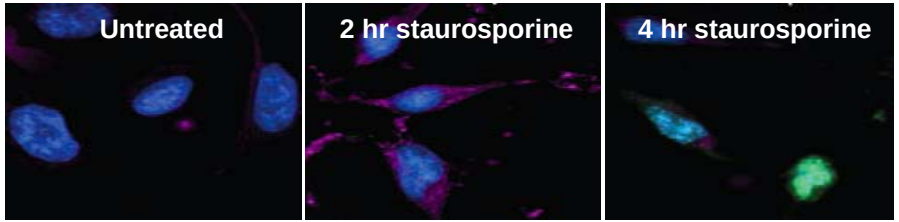
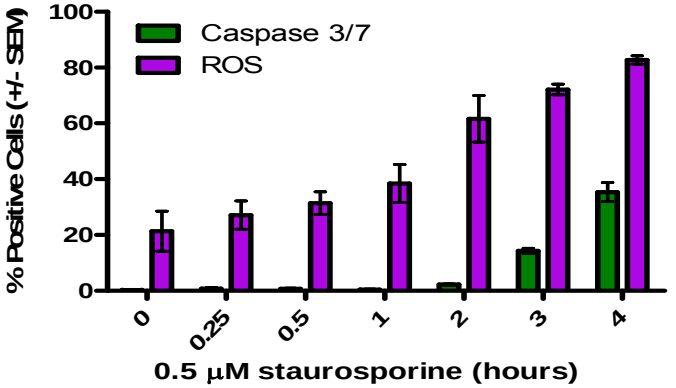
CellROX™ Deep Red Continued



CellROX™ Deep Red, CellEvent Caspase 3/7 Green, and Hoechst reagents added to live HeLa cells in complete media

	Dichlorofluore-scein diacetate	Dihydroethidium	Far Red ROS Sensor
Add in Complete media	No	Yes	Yes
Fixable	No	No	Yes
Multiplexibility	No	No	Yes
GFP compatibility	No	No	Yes



CellROX™ Deep Red Reagent may also be used for flow cytometry, high content screening, and whole-well fluorescence plate reader assays

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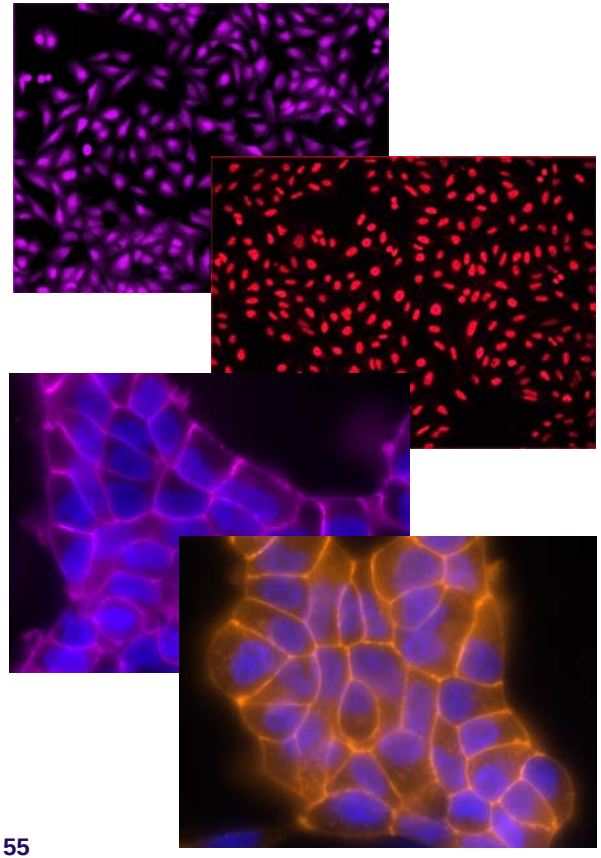
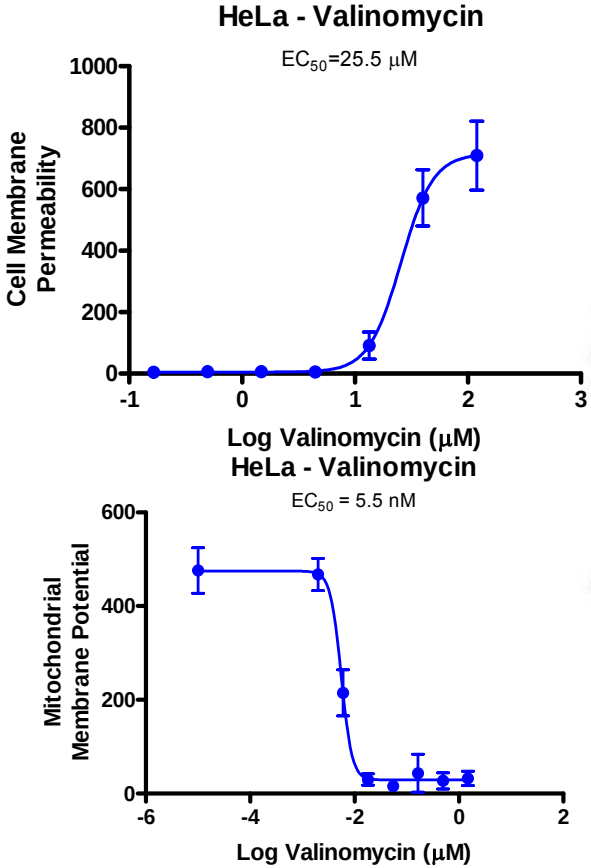
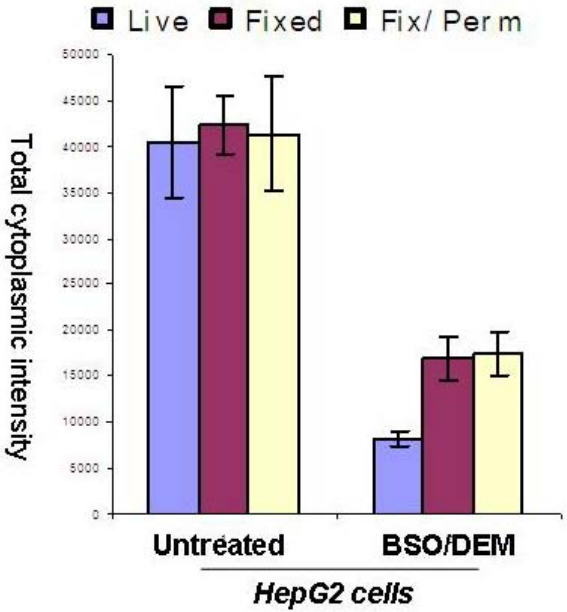
The High Content Imaging Toolbox: Automated Microscopy and Analysis







HCS Segmentation Tools

Staining of Entire (Fixed) Cells

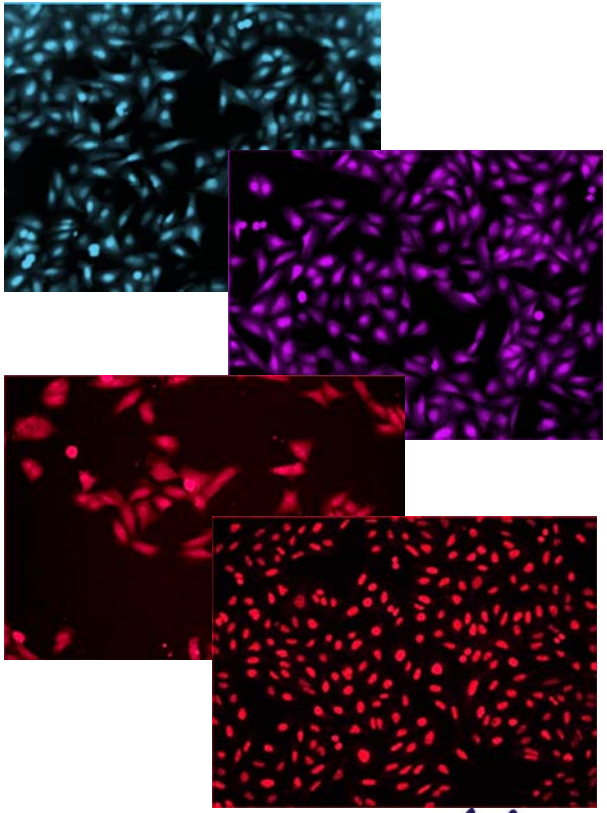
HCS CellMask™ Blue stain
HCS CellMask™ Green stain (new)
HCS CellMask™ Orange stain (new)
HCS CellMask™ Red stain (new)
HCS CellMask™ Deep Red stain

Prominent Nuclear Staining in (Live or Fixed) Cells

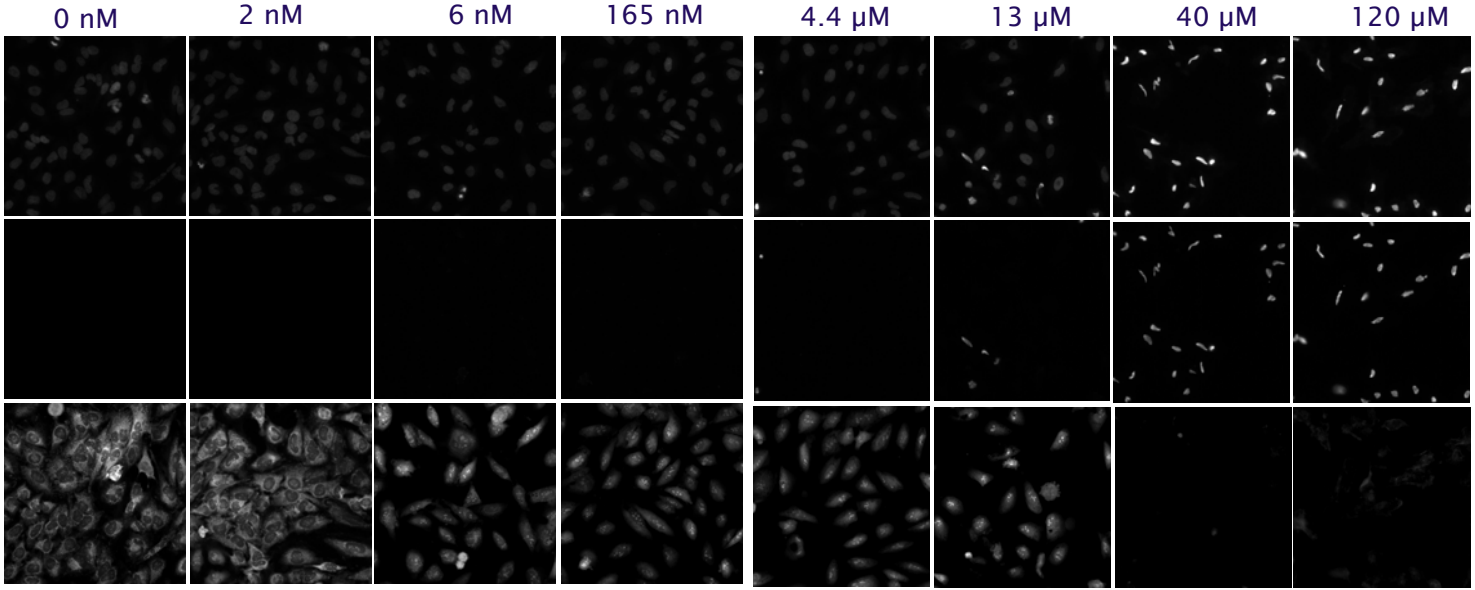
HCS NuclearMask™ Blue stain (new)
HCS NuclearMask™ Red stain (“new”)
HCS NuclearMask™ Deep Red stain

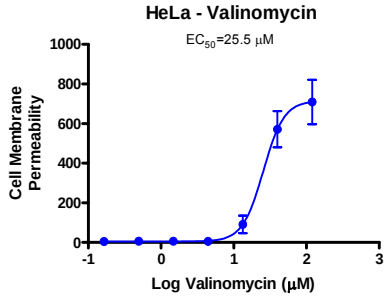
Fixable Plasma Membrane Staining in (Live) Cells

CellMask™ Orange Plasma Membrane stain
CellMask™ Deep Red Plasma Membrane stain



HCS Mitochondrial Health Kit





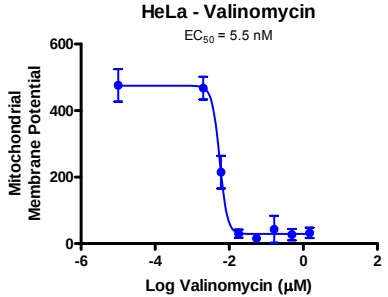
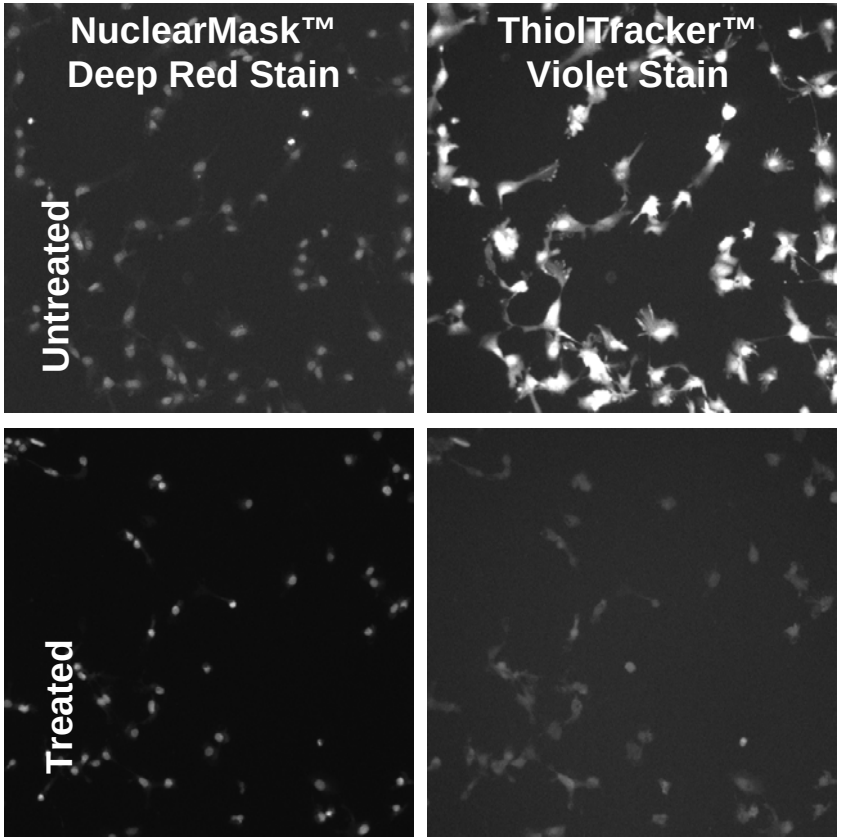


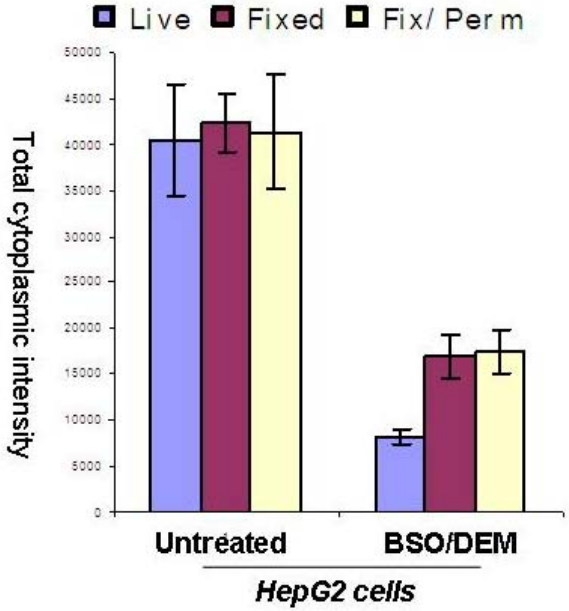
Image-IT® Dead: EC50= 25 uM

MitoHealth: EC50= 5.5 nM

ThiolTracker™ Violet for Glutathione Depletion Imaging



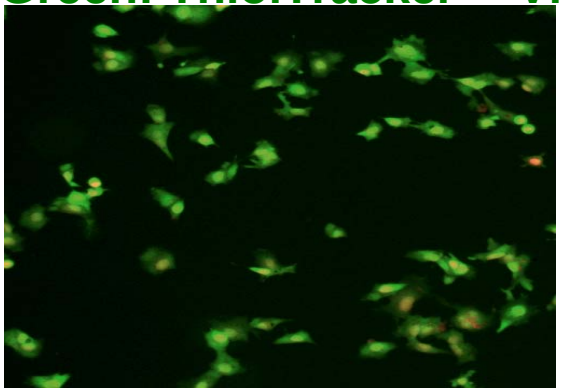
Live HepG2 cells



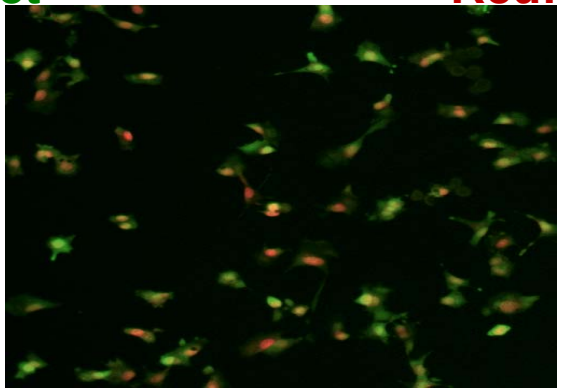
Glutathione Depletion

Green: ThiolTracker™ Violet

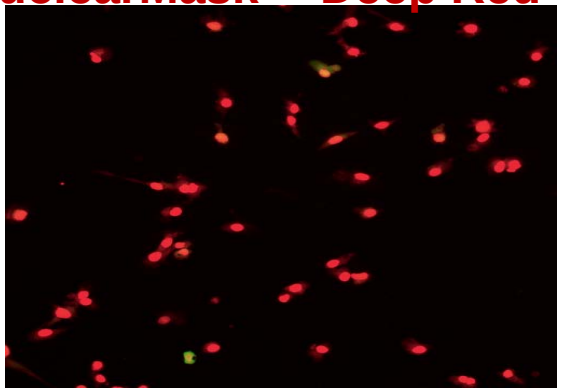
Red: NuclearMask™ Deep Red



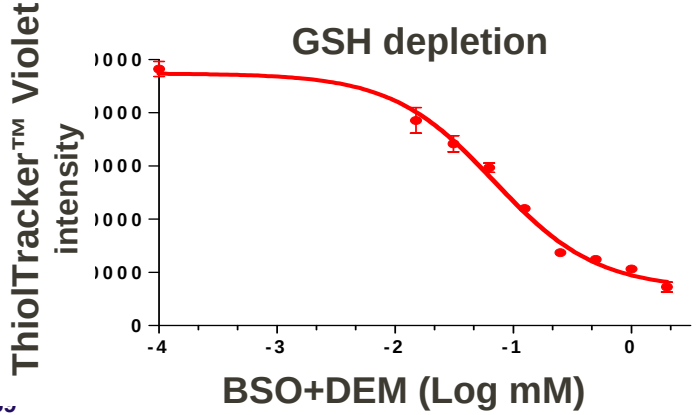
Control



125 µM BSO + 62.5 µM DEM



1 mM BSO + 0.5 mM DEM



Sensitive assay for signature cytotoxicity marker (GSH)

- Effective marker for intracellular reduced glutathione (GSH)
- Easy to use and fixable
- Deep red nuclear stain ideal for multiplexing
- Much brighter than bimanes

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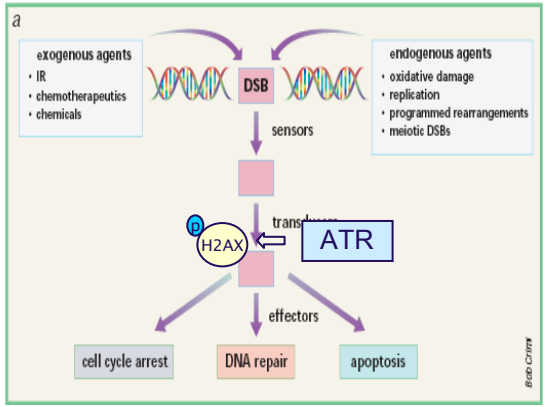
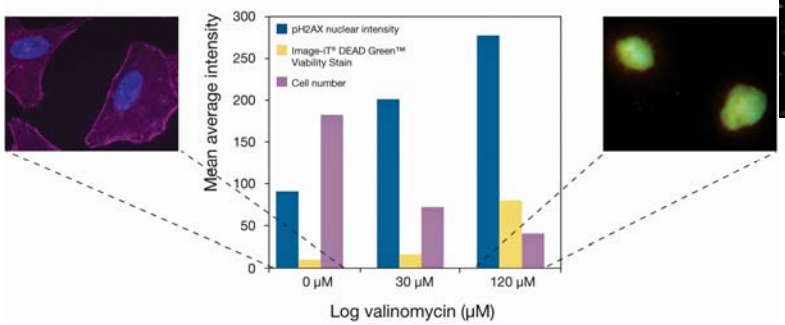
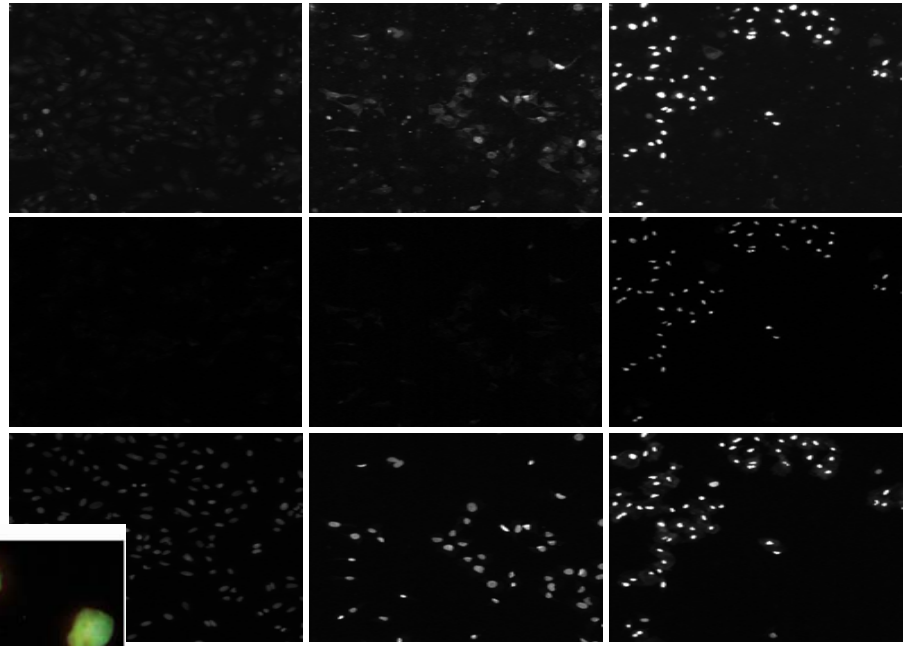
DNA Damage by phospho-H2AX Staining



Khanna, K.K. and Jackson, S.P. (2001)
Nature Genet., 27, 247-254



DMSO | 30 µM | 120 µM



A549 Cells
24h Valinomycin

DNA damage
(pH2AX antibody/
Alexa Fluor® 555 secondary)

Cell Membrane Permeability
(Image-iT® DEAD Green™
viability stain)

Nuclear morphology
(Hoechst 33342)

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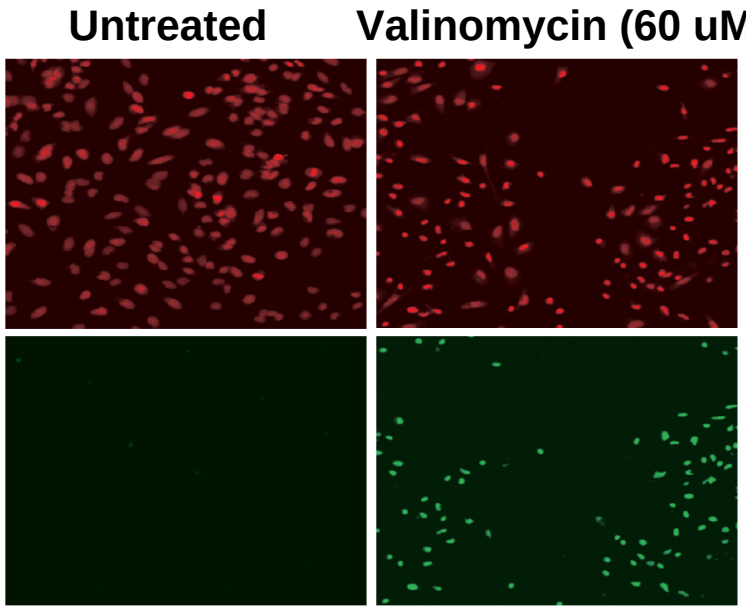
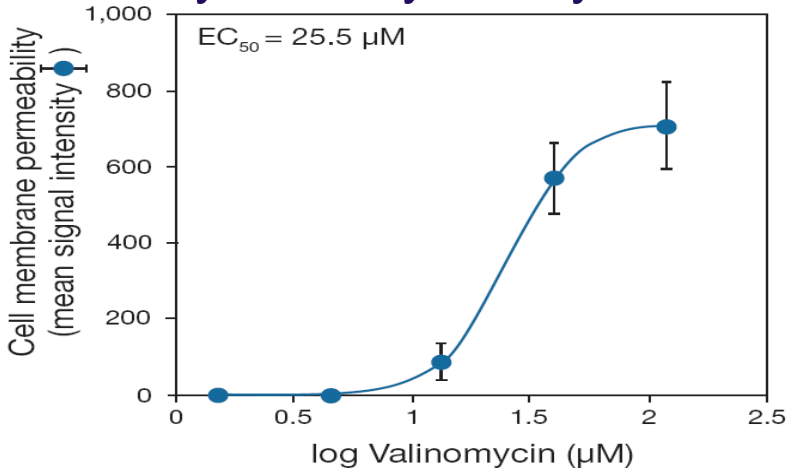
HCS LIVE/DEAD® Green Kit



Nuclear morphology (HCS Nuclear Mask™ Deep Red stain)

Cell membrane permeability (Image-iT® DEAD Green viability stain)

Fast and highly sensitive two-color cytotoxicity assay



- Dye-based, two color cytotoxicity assay with fast and efficient workflow
- Sensitive discrimination of live and dead cells
- Compatible with fixation and permeabilization for combination with antibody-based assays

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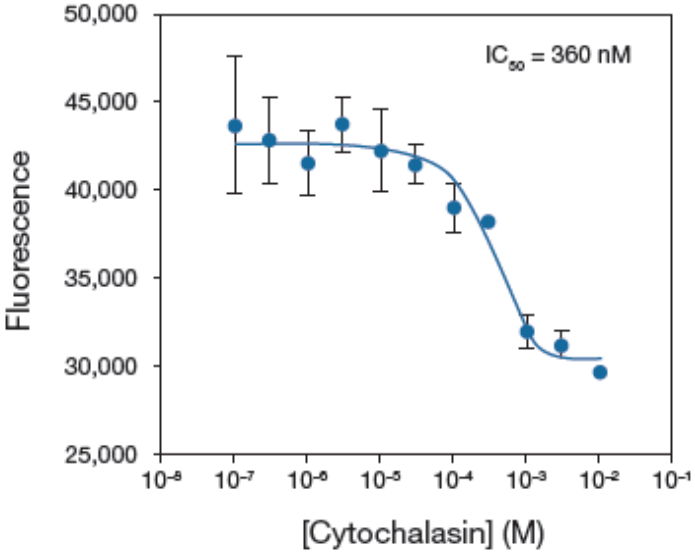
Live Cell HCS with pHrodo™ e. coli

ImageXpress Micro®



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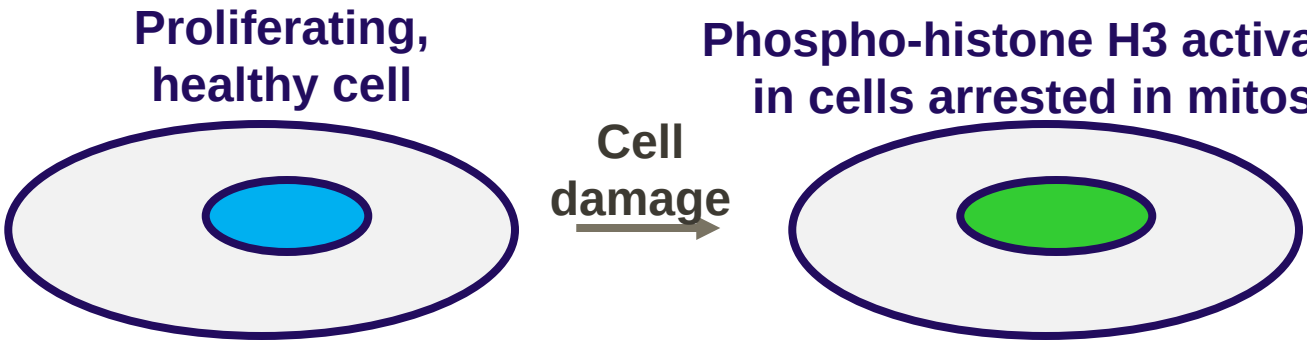
HCS Mitotic Index Kit



Measures % of mitotic cells

- Phospho-histone 3 is a known mitotic marker, commonly used in cancer research, toxicology and cell cycle signaling
- Phospho-histone H3 primary with Alexa Fluor® 488 secondary enables characterization and quantitation of mitotic cells and compounds causing mitotic arrest
- Kit contains DAPI or HCS NuclearMask™ Deep Red for total DNA content
- Can be combined with other cell cycle markers to study cell cycle related and other physiological pathways
- Sensitive and accurate assay for mitotic arrest and toxicity

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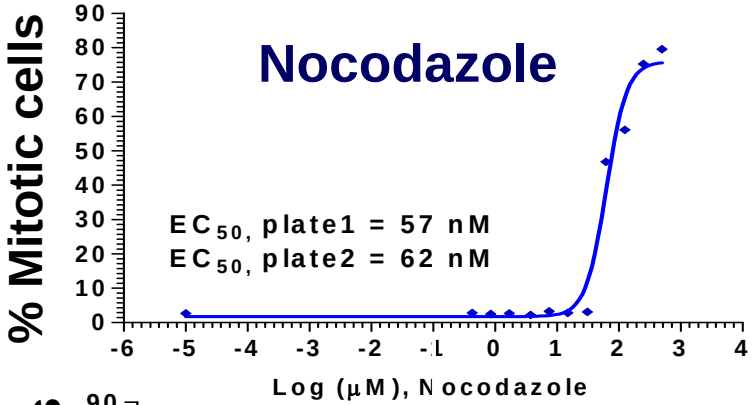
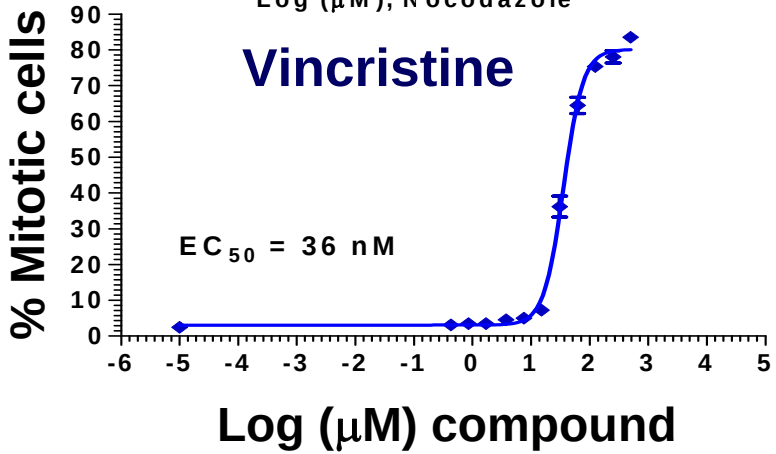
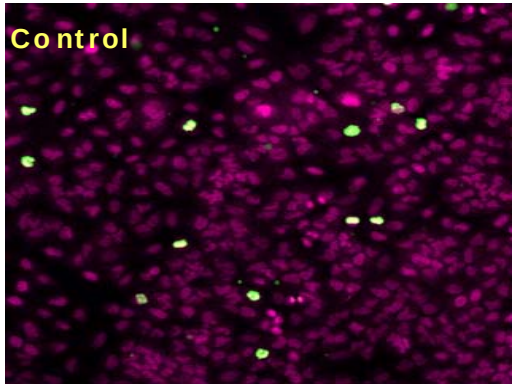
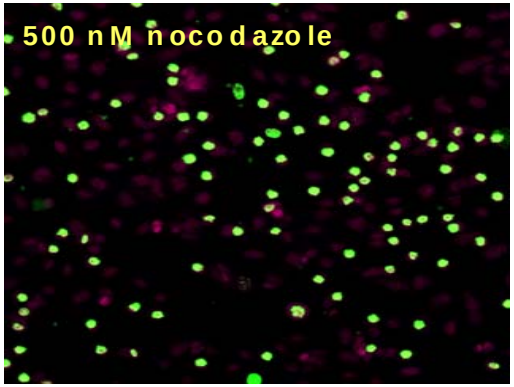
HCS Mitotic Index Kit







HCS NuclearMask™ Deep Red Stain



phospho-H3 Ab / Alexa Fluor® 488 secondary

Excellent assay robustness with tight CV values and Z' scores

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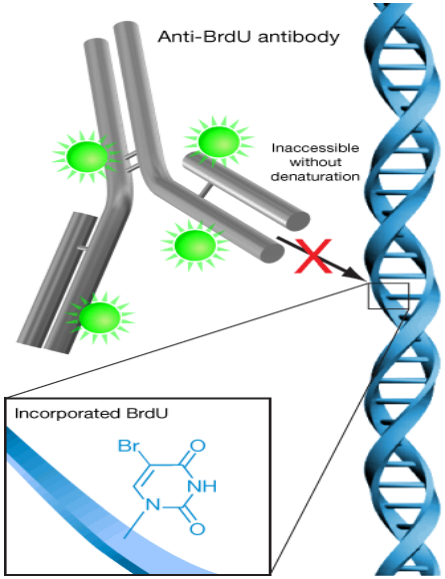
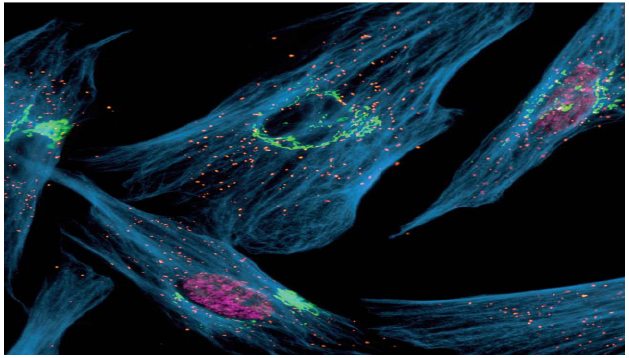
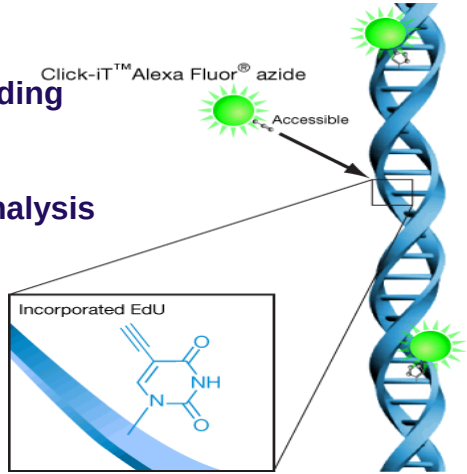
Breakthrough cell proliferation assay with Click-iT® EdU





- Non-radioactive
- No DNA denaturation required
- Simplified protocol
- Small molecule detection
- Multiplex compatible, including
  - Other antibodies
  - Dyes for cell cycle analysis



- Non-radioactive
- Multiplex compatible *but*, strand separation requirement for anti-BrdU access, can affect:
  - Ability for other antibodies to bind
  - Morphology
  - Ability for dyes that require dsDNA to bind efficiently, i.e., cell cycle dyes

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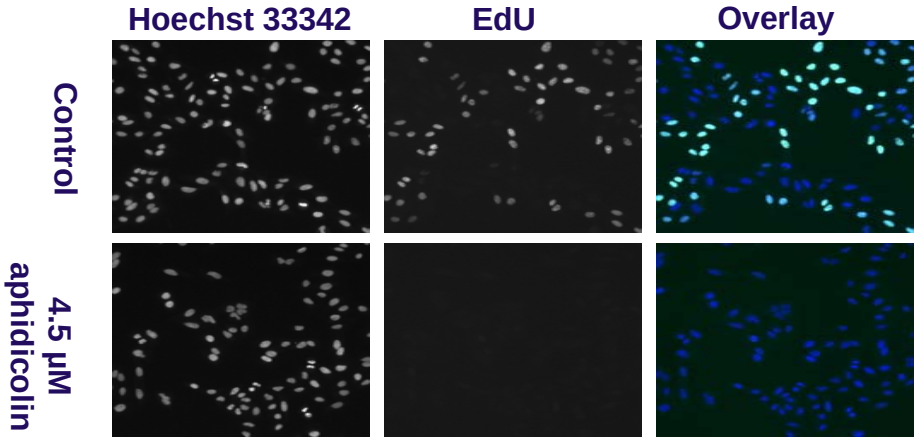
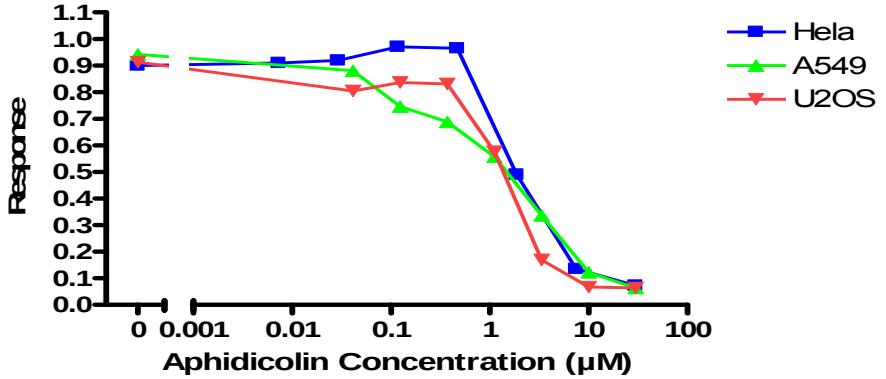
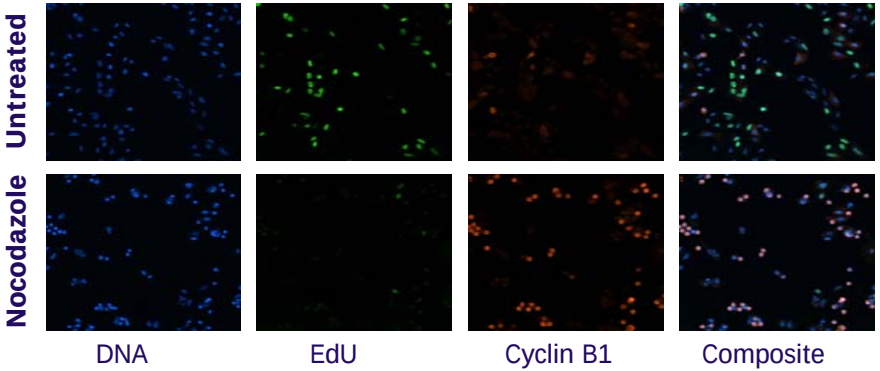
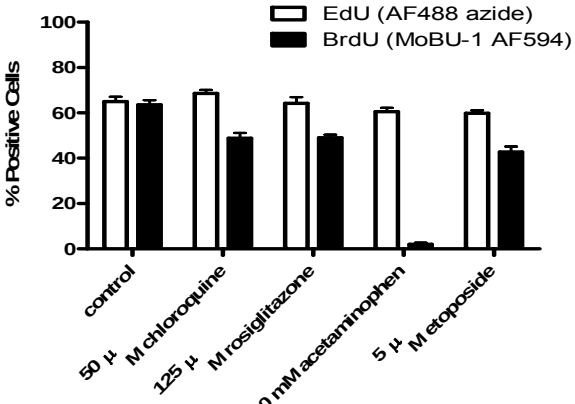
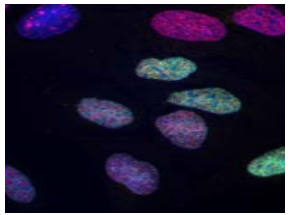
HCS Click-iT® EdU assay









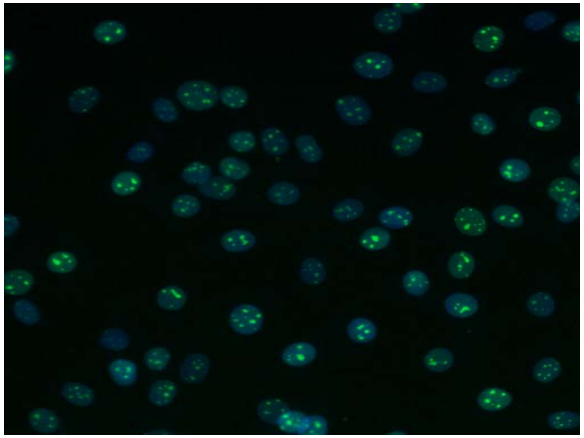


Cells fed with 10µM Edu

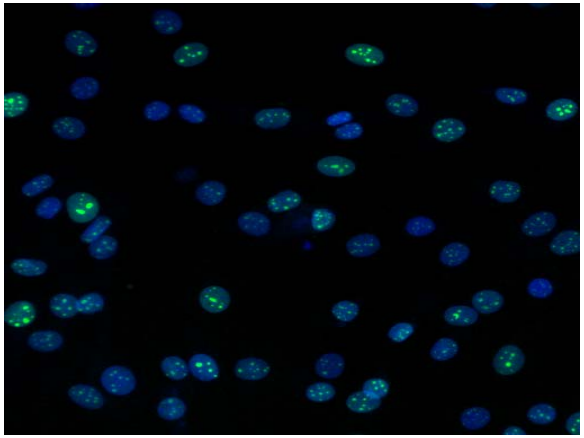
Drug treatment-23 hrs

Cells fed with 10µM BrdU

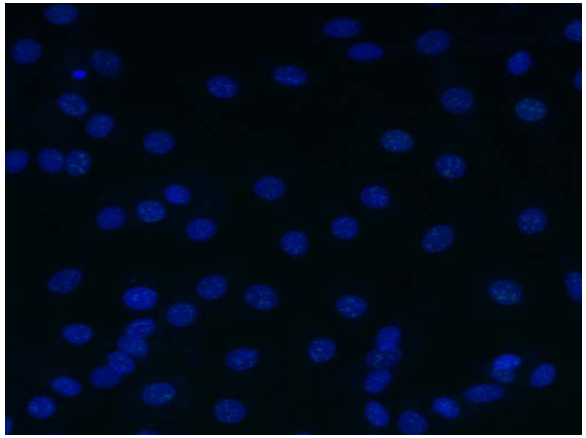
Nascent RNA synthesis block measured with HCS Click-iT® EU Assay



α-Amanitin 0nM

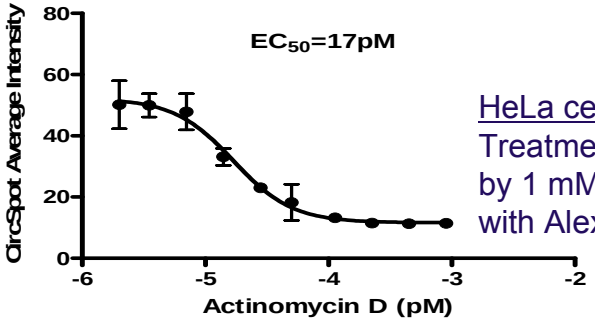


α-Amanitin 30nM



α-Amanitin 250nM

NIH 3T3 cells (images)
Treatment with α-Amanitin for 18 hr followed by 1 mM EU incubation for 1 hr, click rxn with Alexa Fluor® 488 azide (green) and nuclear counterstaining with Hoechst (blue).



HeLa cells (dose response)
Treatment with actinomycin for 18 hr followed by 1 mM EU incubation for 1 hr and click rxn with Alexa Fluor® 488 azide

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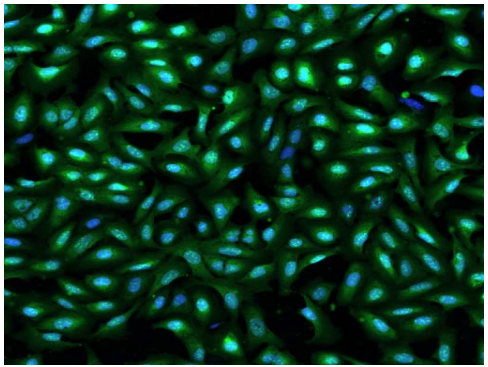
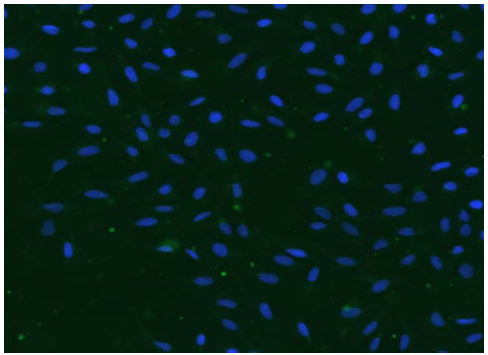
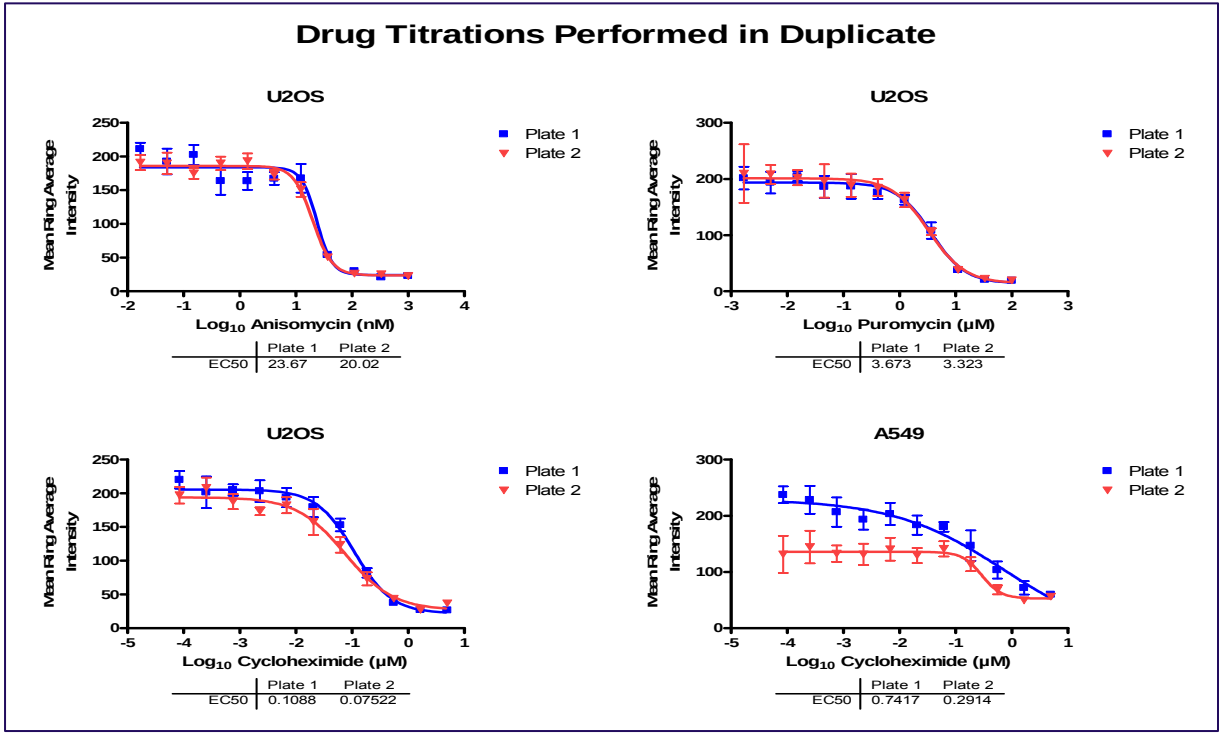
Nascent Protein Imaging with HCS Click-iT® AHA







- Validated HCS kit that enables detection of pre-lethal effects of compounds on nascent protein synthesis
- Faster and safer alternative to radioactive methionine techniques

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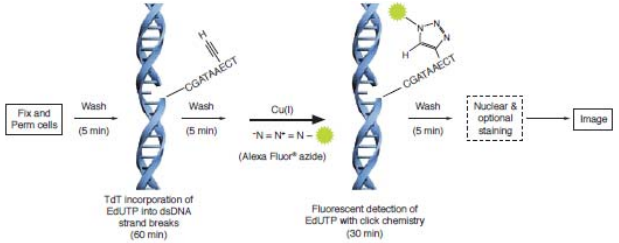
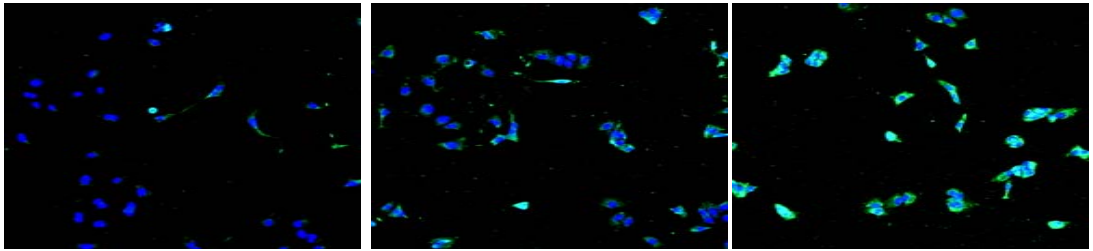
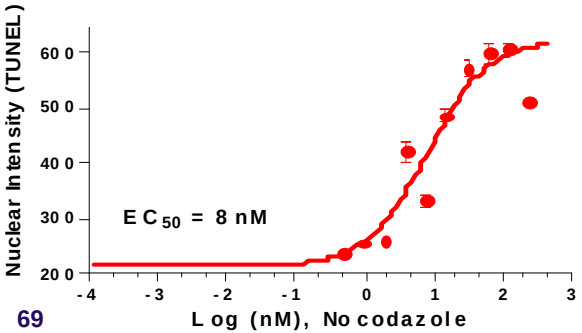
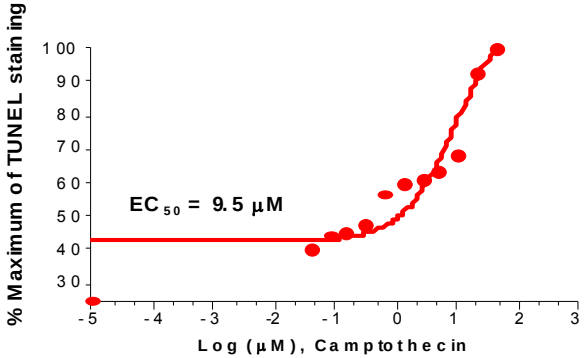
Apoptotic HeLa cells in the HCS Click-iT® TUNEL Assay

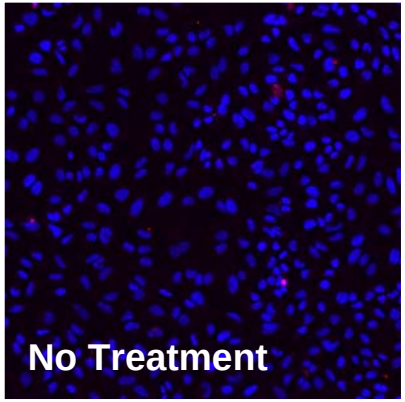


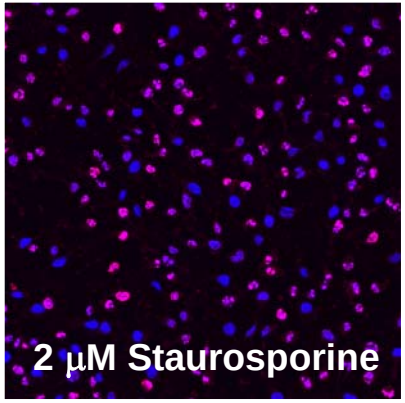


Control | 5 µM Camptothecin | 40 µM Camptothecin









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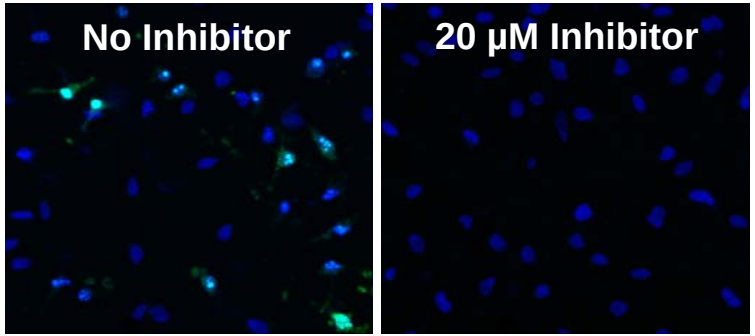
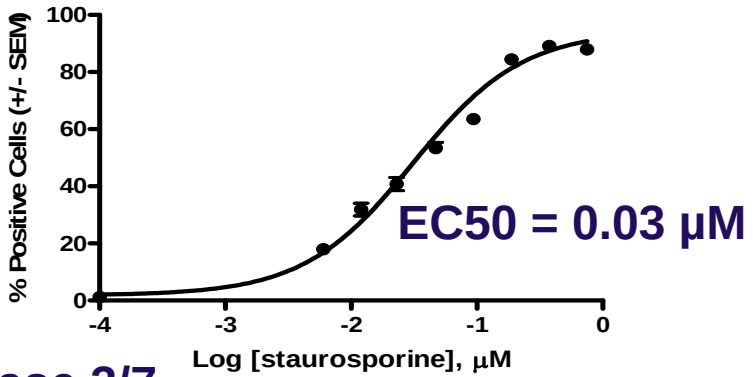
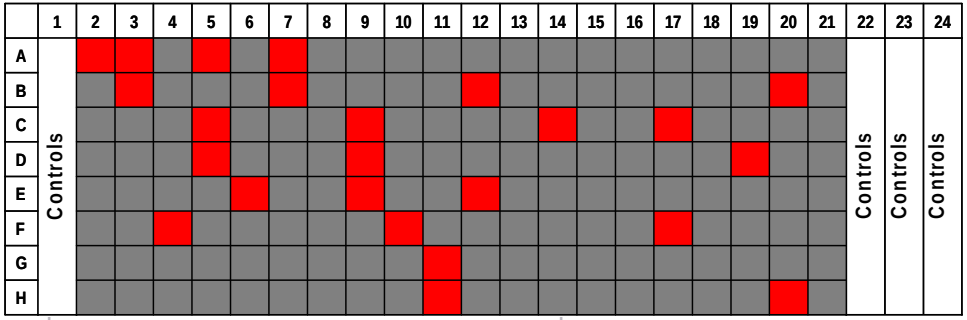
Apoptosis in HCS with CellEvent™ Caspase 3/7 Reagent

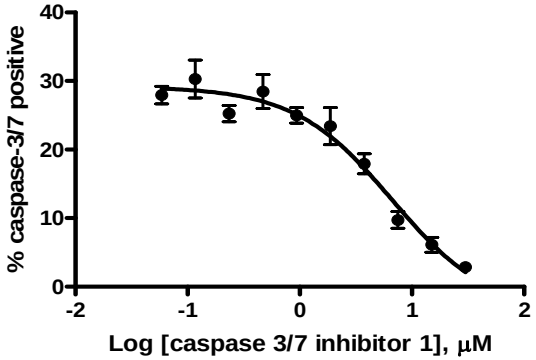
CellEvent™ Green Fluorogenic Caspase 3/7 substrate





High content screening for activation of caspase 3/7





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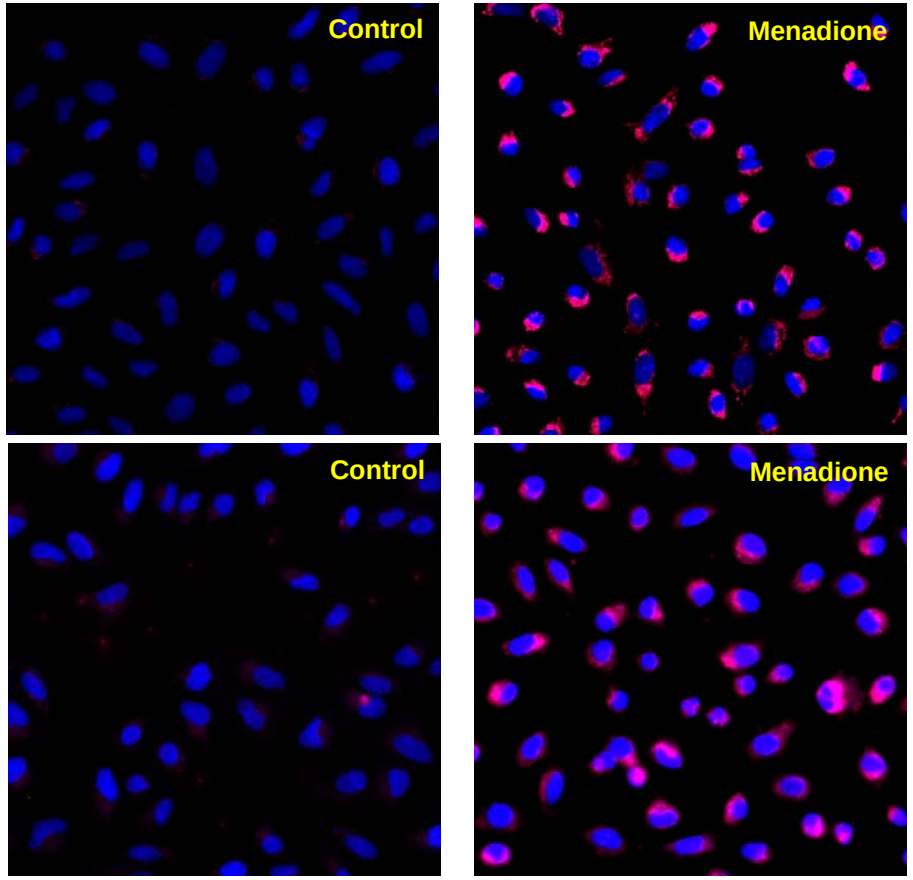
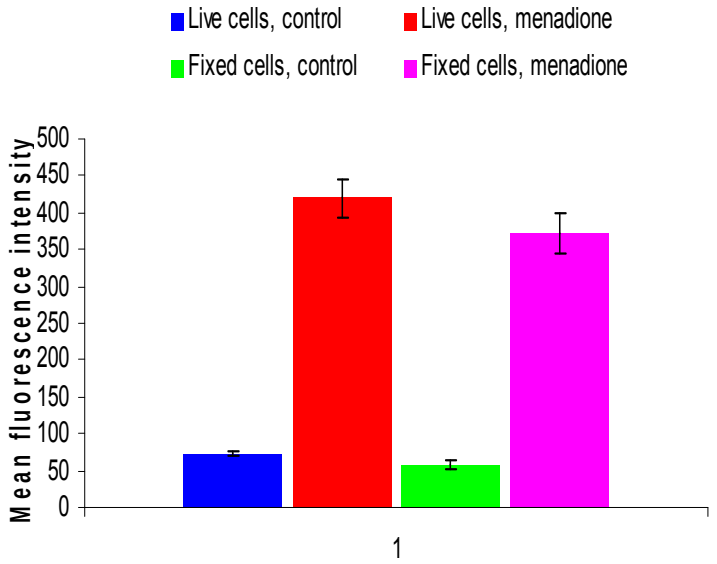
CellROX™ Deep Red ROS Sensor in Live and Fixed





U-2 OS Cells

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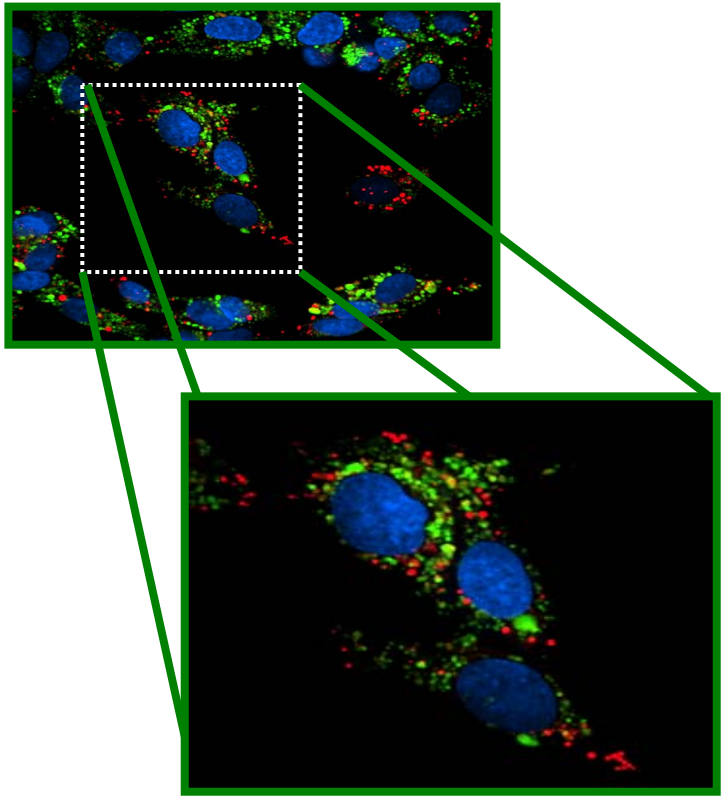
HCS LipidTOX™ phospholipidosis and steatosis

HepG2 cells,
10 μM amiodarone
48 hours



Kits include:

LipidTOX™ Red phospholipid stain

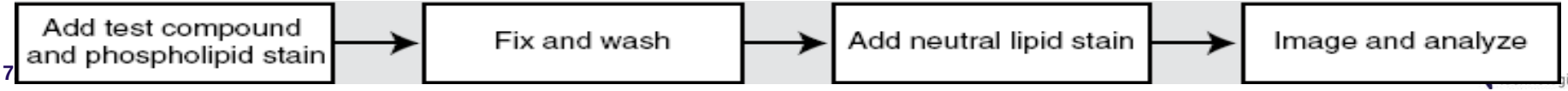
LipidTOX™ Green neutral lipid stain

Propranolol

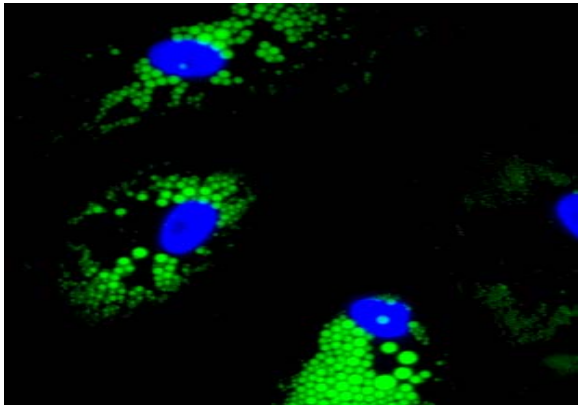
Cyclosporin A

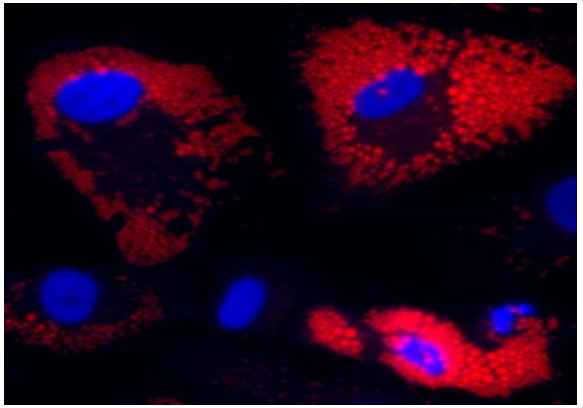
LipidTOX™ probes also available as "stand alone" reagents

Hoechst 33342

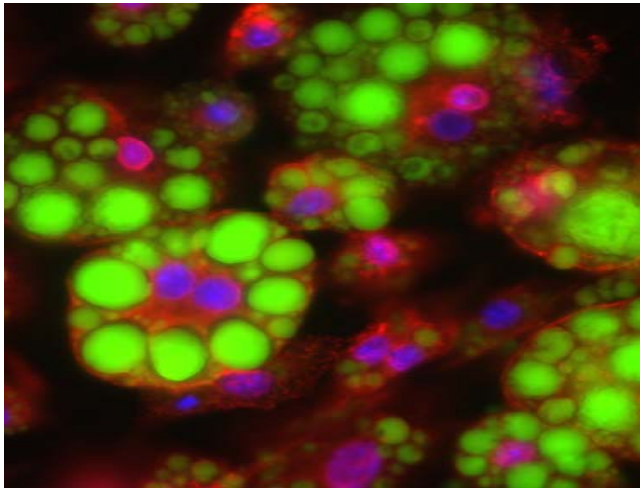


LipidTOX™ neutral lipid stains for adipogenesis





Adipocytes differentiated from human mesenchymal stem cells, fixed, and labeled with LipidTOX™ Green or Deep Red neutral lipid stain and Hoechst 33342



Adipocytes differentiated from 3T3 L1 mouse fibroblasts, fixed, and labeled with LipidTOX™ Green neutral lipid stain, anti-FABP4 (red), and Hoechst 33342

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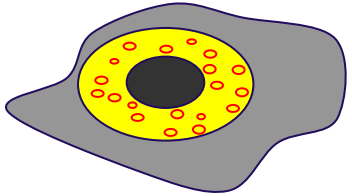
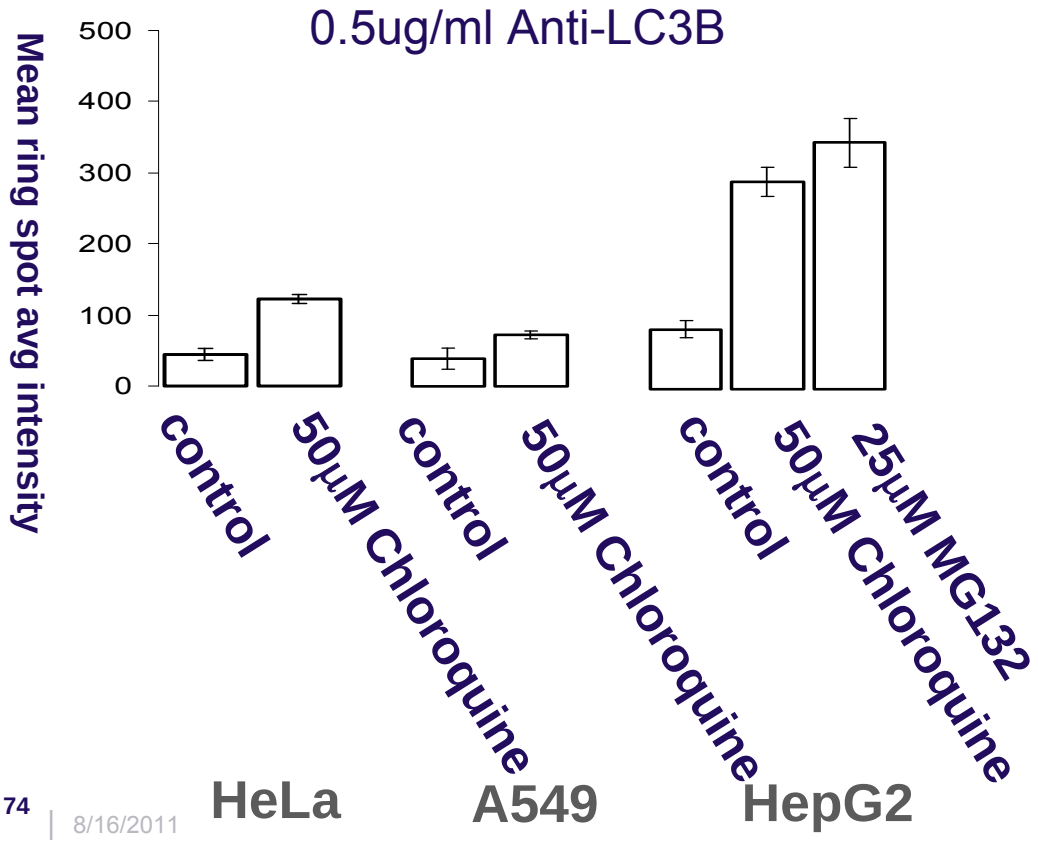
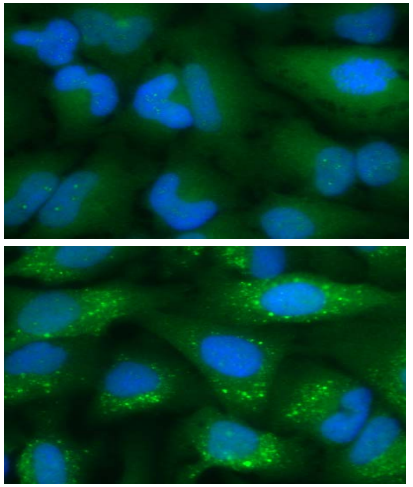
HCS Immunodetection of LC3B







http://www.invitrogen.com/hcs

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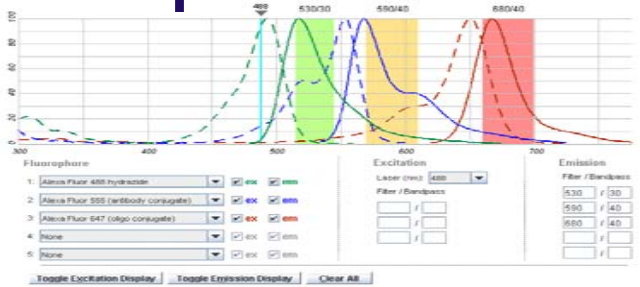
Web-based Accessories

Spectra Viewer

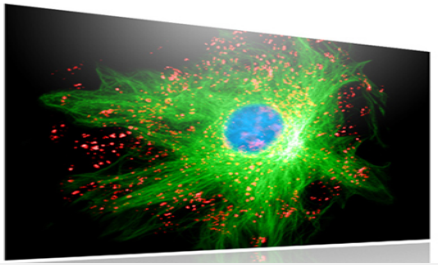


www.invitrogen.com/spectraviewer

The Virtual Cell

Stain Your Own Cell

molecular probes | invitrogen



www.invitrogen.com/cellstaintool

Facebook Molecular Probes Handbook Club

10,000+ Members since Dec 2010

https://www.facebook.com/cellimaging

Facebook Cell Imaging

25,000+ Members since Dec 2010

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Questions? techsupport@lifetech.com / 800.955.6288

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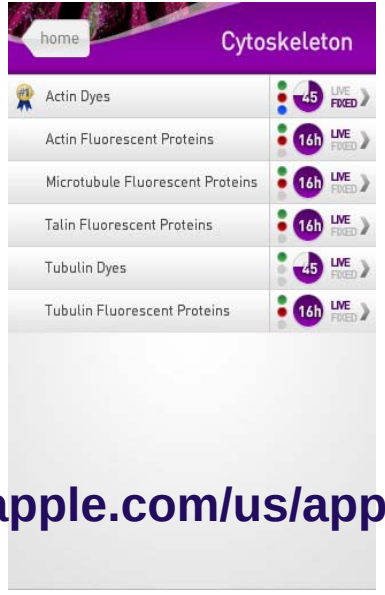
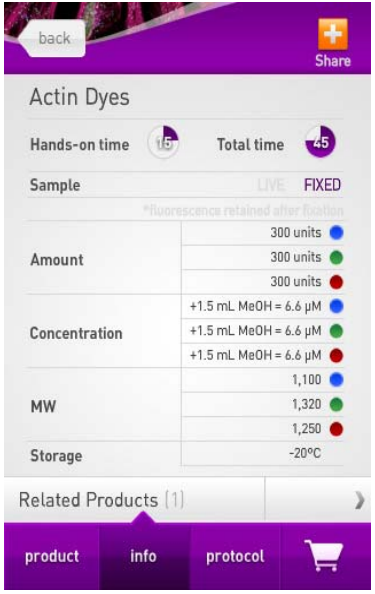
Cell Imaging App for iPhone, iPad and Android

Cell Features and Biology



Reagents and Results



Protocols and Info

http://itunes.apple.com/us/app/cell-imaging

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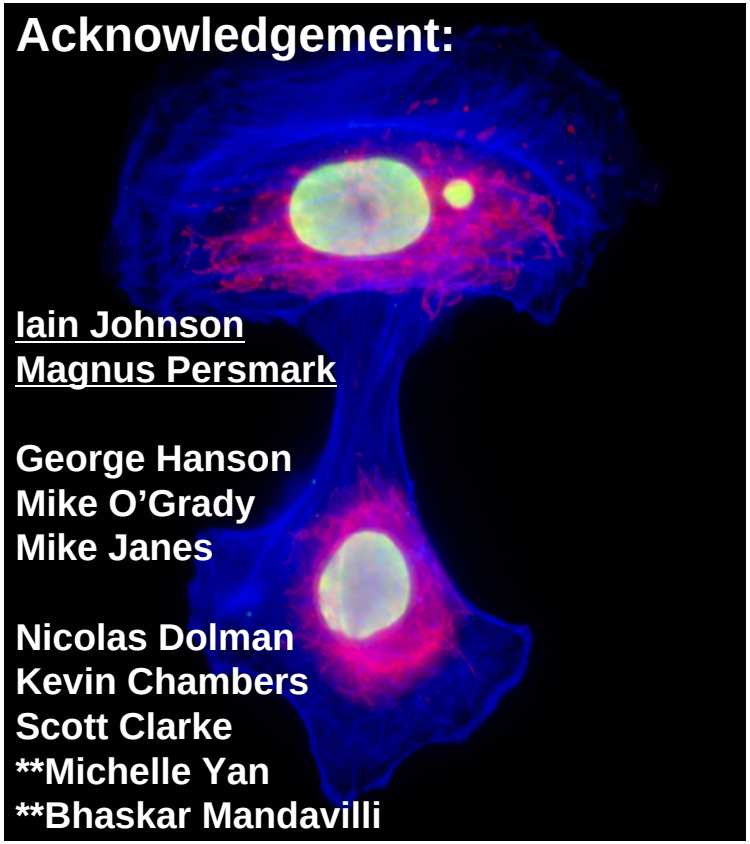
Summary

- Molecular Probes is alive and well!!

- New dyes and new chemistry
  - Would like your input

- Fluorescent Proteins + BacMam
  - = EASY!
  - Would like your content

Questions?



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