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
Dyes, Indicators and Affinity Labels

**Fluorescent Dyes**

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

**Alexa Fluor® Dyes**

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

**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⁻¹cm⁻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⁻¹cm⁻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
Probing Compartmental pH with pHrodo™ Dyes
Phagocytic Index with pHrodo™ Dye

DeltaVision™ Core
pHrodo™ Dextran for Endocytosis and Sorting

![Diagram showing endocytosis and sorting pathways.]

- CV
- Early Endosome
- Rab 5
- Early Endosome Rab 5/4
- pH 6
- Rab7/9
- Recycling Endosome Rab 4/11
- Late Endosome

![Images showing fluorescence microscopy results for Vehicle and Dynasore treated cells.]

- Vehicle
- Dynasore

![Graph showing IC50 Dynasore at 4.7 μM.]

IC50 Dynasore 4.7 μM
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
**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
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
**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
Click-iT® Chemistry for Modular Detection

Simple Multiplexing
Expand the boundaries of your Discovery Research

- Easy to Use - No expertise needed
- Fast - Reduce the amount of time required
- Save Money & Labor - Fewer experiments required

Simple click reactions use biologically unique and inert moieties to label and eventually detect a molecule.

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

Nascent DNA Synthesis
**Click-iT® Chemical Biology**

Azide + Cu(I) at Room Temp → Triazole

DNA + RNA + Protein + PTM → Substrate

<table>
<thead>
<tr>
<th></th>
<th>Radioactivity</th>
<th>Click-iT™ alkyn</th>
<th>Click-iT™ azide</th>
<th>Biotin</th>
<th>Alexa Fluor® 488</th>
<th>Streptavidin</th>
<th>IgG antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>0</td>
<td>21</td>
<td>42</td>
<td>~300</td>
<td>500</td>
<td>~68,000</td>
<td>~150,000</td>
</tr>
</tbody>
</table>

Both required for detection
Click-iT® EdU: Proliferation on Single Cell Level

BrdU

Click-iT® EdU

Anti-BrdU antibody
Inaccessible without denaturation

Click-iT™ Alexa Fluor® azide
Accessible

Incorporated BrdU

Incorporated EdU
Click chemistry EdU Protocol

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Click-iT™ detection reaction</td>
<td>30 min</td>
</tr>
<tr>
<td>Wash 2X</td>
<td>10 min</td>
</tr>
<tr>
<td>Nuclear counterstain</td>
<td>15 min</td>
</tr>
<tr>
<td>Wash 3X</td>
<td>15 min</td>
</tr>
<tr>
<td>Image</td>
<td>TOTAL TIME 70 minutes</td>
</tr>
</tbody>
</table>

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)

BrdU Protocol

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash 3X</td>
<td>15 min</td>
</tr>
<tr>
<td>HCl Denaturation</td>
<td>40 min</td>
</tr>
<tr>
<td>Neutralize</td>
<td>12 min</td>
</tr>
<tr>
<td>Wash 3X</td>
<td>15 min</td>
</tr>
<tr>
<td>Block</td>
<td>60 min</td>
</tr>
<tr>
<td>Anti-BrdU incubation</td>
<td>1-16 hours</td>
</tr>
<tr>
<td>Wash 3X</td>
<td>15 min</td>
</tr>
<tr>
<td>Secondary antibody incubation</td>
<td>120 min</td>
</tr>
<tr>
<td>Wash 3X</td>
<td>15 min</td>
</tr>
<tr>
<td>Nuclear counterstain</td>
<td>15 min</td>
</tr>
<tr>
<td>Wash 3X</td>
<td>15 min</td>
</tr>
</tbody>
</table>
| Image                       | TOTAL TIME 21 hours
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

Rat Ileum

Rat Brain

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)


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

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

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

zebrafish larva
Image courtesy of Sarah Cheesman, University of Oregon

E. coli
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).
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)
Click-iT® Nascent Protein Synthesis

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

Click-iT® TUNEL Assay for DNA Damage

Primary human hepatocytes

Hoechst  Click-iT® TUNEL  Overlay

Stress/Ox DNA Nicking

Control  10 µM Actinomycin D
Click-iT® Detection Assays...

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

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

Nascent RNA Synthesis
Click-iT® RNA Assay

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

www.invitrogen.com/clickit
# 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

Azide-modified molecule + Fluorophore-, or hapten-DIBO → Stable triazole conjugate

DIBO Alkynes react with incorporated azide macromolecules
Copper-Free Click Mannosylation in GFP Cells

Label:
ManNAz Azido Mannose Label

Detect:
Alexa Fluor® 647 DIBO alkyne
BacMam Gene Delivery

Targeted fluorescent proteins + BacMam

CellLight® Reagents

Premo™ Biosensors

BacMam Targets: Ion Channel, GPCR, Kinase etc
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

---

**Diagram:**

1. Recombinant baculovirus DNA
2. SF9 cells
3. Transduce overnight
4. BacMam virus
5. Assay
How Do I Use BacMam?

Cells + BacMam reagent

Incubate overnight at 37°C

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

Tubulin GFP

Actin GFP
Actin Stress Fiber Disruption

Cytochalasin D

Dissolves actin filaments
Tubulin network intact

Tubulin GFP
Actin RFP
Hippocampal Neurons

- GFP Control
- MAP4 RFP
- Actin RFP
- GFP + Mito RFP
- Synaptophysin-RFP
Growth Cone Dynamics and Mito Traffic

GFP Control
Mito RFP
Premo™ Cameleon Calcium Sensor
Premo™ Halide Sensor

[Diagram showing the process of Baculovirus infection and gene expression]

[Graph showing changes in ΔF over time]
FUCCI Cell Cycle Sensor

GFP-Geminin
+ RFP Cdt1
= FUCCI

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
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
Premo™ LC3B Autophagy Sensor

Vehicle                   Chloroquine

LC3B

Mutant

Vehicle                   Chloroquine
Autophagy in Primary Animal and Human Cells

Rat Hippocampal

LC3B G120A

WT LC3B

Human Aortic Smooth Muscle Cells

BacMam LC3B-GFP + CellLight® Golgi-RFP
Rhod-3 Calcium Imaging with CaV2.1

$4X \Delta F/F$

$+30 \text{ mM K}^+$
$+15 \text{ mM K}^+$
$+5 \text{ mM K}^+$

1 min

BacMam CaV2.1 $\alpha + \beta + \alpha 2\delta$

Kd 570 nM
CellEvent™ Caspase 3/7 Green Apoptosis Sensor

Active Caspase-3/7 Enzyme

Non-fluorescent
No DNA binding

Fluorogenic signal upon DNA binding
CellEvent™ Caspase 3/7 Imaging

A  CellEvent™

B  Hoechst

C  Staurosporine

D  AF 647 Phalloidin Mitotracker Red

AF 647 Phalloidin Mitotracker Red Hoechst
CellEvent™ Caspase 3/7 Time Lapse in HCS

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 fluorescence 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.

![Chemical structure](image)

Reduced (Nonfluorescence) → Oxidized (Fluorescence)
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
CellROX™ Deep Red ROS Sensor

Live cells
Control
Menadione

Fixed cells
Control
Menadione

Mean fluorescence intensity

- Live cells, control
- Live cells, menadione
- Fixed cells, control
- Fixed cells, menadione

U-2 OS Cells
Reactive Oxygen Imaging

Control

100 µM Menadione

Hoechst/CellROX™ Deep Red reagent

BPAE Cells
**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.
CellROX™ Deep Red Reagent may also be used for flow cytometry, high content screening, and whole-well fluorescence plate reader assays.
The High Content Imaging Toolbox: Automated Microscopy and Analysis

HeLa - Valinomycin

EC_{50} = 25.5 μM

EC_{50} = 5.5 nM

Mitochondrial Membrane Potential

Cell Membrane Permeability

Total cytoplasmic intensity

HepG2 cells

Untreated BSO/DEM

Live Fixed Fix/Perm
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

<table>
<thead>
<tr>
<th>Concentration (nM/μM)</th>
<th>0 nM</th>
<th>2 nM</th>
<th>6 nM</th>
<th>165 nM</th>
<th>4.4 μM</th>
<th>13 μM</th>
<th>40 μM</th>
<th>120 μM</th>
</tr>
</thead>
</table>

**Hoechst 33342**
(Nuclear Morphology)

**Image-IT® DEAD Green™ Viability stain**
(Cell Membrane Permeability)

**MitoHealth stain**
(Mitochondrial Membrane Potential)

**HeLa - Valinomycin**

<table>
<thead>
<tr>
<th>Log Valinomycin (μM)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50</td>
<td>25.5 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Image-IT® Dead:**
EC50 = 25 μM

**MitoHealth:**
EC50 = 5.5 nM
ThiolTracker™ Violet for Glutathione Depletion Imaging

NuclearMask™ Deep Red Stain

ThiolTracker™ Violet Stain

Untreated

Treated

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
DNA Damage by phospho-H2AX Staining


<table>
<thead>
<tr>
<th>DMSO</th>
<th>30 µM</th>
<th>120 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**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)

**Graphical Data**

![Graph](graph.png)
HCS LIVE/DEAD® Green Kit

<table>
<thead>
<tr>
<th>Untreated</th>
<th>Valinomycin (60 uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Untreated" /></td>
<td><img src="image2" alt="Valinomycin" /></td>
</tr>
</tbody>
</table>

**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

![Graph](image3)

$EC_{50} = 25.5 \text{ } \mu\text{M}$
Live Cell HCS with pHrodo™ e. coli

ImageXpress Micro®

![ImageXpress Micro®](image)

![Graph showing fluorescence vs. [Cytochalasin] (M)](graph)

\[ IC_{50} = 360 \text{ nM} \]
HCS Mitotic Index Kit

- 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
HCS Mitotic Index Kit

Excellent assay robustness with tight CV values and Z’ scores

**Nocodazole**

EC<sub>50</sub>, plate 1 = 57 nM
EC<sub>50</sub>, plate 2 = 62 nM

**Vincristine**

EC<sub>50</sub> = 36 nM

Log (μM) compound

HCS NuclearMask™
Deep Red Stain

phospho-H3 Ab / Alexa Fluor® 488 secondary
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
HCS Click-iT® EdU assay

Hoechst 33342  EdU  Overlay

Control

4.5 µM aphidicolin

A549  U2OS

0.001  0.1  1  10  100

Aphidicolin Concentration (µM)

Response

DNA  EdU  Cyclin B1  Composite

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

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

EC₅₀=17pM
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
Apoptotic HeLa cells in the HCS Click-iT® TUNEL Assay

EC₅₀ = 8 nM

EC₅₀ = 9.5 µM
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

EC50 = 0.03 μM
CellROX™ Deep Red ROS Sensor in Live and Fixed Menadione

Live cells

- Control
- Menadione

Fixed cells

- Control
- Menadione

Mean fluorescence intensity

- Live cells, control
- Live cells, menadione
- Fixed cells, control
- Fixed cells, menadione

U-2 OS Cells
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
Hoechst 33342

*LipidTOX™ probes also available as “stand alone” reagents
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
HCS Immunodetection of LC3B

Mean ring spot avg intensity

0.5μg/ml Anti-LC3B

control
50μM Chloroquine
control
50μM Chloroquine
control
50μM Chloroquine
25μM MG132

HeLa
A549
HepG2

8/16/2011
http://www.invitrogen.com/hcs
Web-based Accessories

Spectra Viewer

www.invitrogen.com/spectraviewer

Facebook
Molecular Probes
Handbook Club

10,000+ Members since Dec 2010
https://www.facebook.com/ProbesHandbook

Facebook
Cell Imaging

25,000+ Members since Dec 2010
https://www.facebook.com/cellimaging

Questions? techsupport@lifetech.com / 800.955.6288

www.invitrogen.com/ cellstaintool
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?

daniel.beacham@lifetech.com