

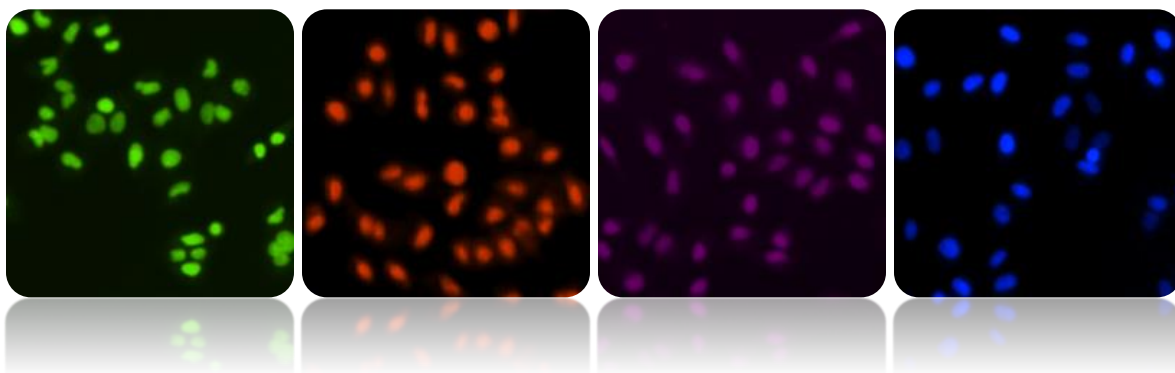


ViaStain™ Live Cell Nuclear Dyes

Product Number:

- CS1-V0008-1 Green
- CS1-V0009-1 Red
- CS1-V0010-1 Far Red
- CS1-V0011-1 Blue

Sample Kit(s): Available for all Live Cell Nuclear Dyes



This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use.

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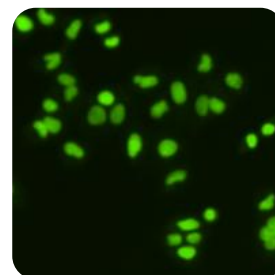
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1.0 Introduction

1.1 Description of Assay

Live Nuclear Dye:

The total number of nucleated cells are identified by staining the cells with Live Nuclear Dye. This is a live DNA binding agent that is membrane permeable and is used to identify all nucleated cells. After staining the cells, the Celigo instrument can scan, analyze and report the number of total cells on a per well basis.



1.2 Materials Included

- Live Nuclear Dye, Store at -20°C

Product Name	Catalog No.	Stock	Volume	No. 96-well Plates
ViaStain™ Live Cell Nuclear Green	CS1-V0008-1	5 mM	100 µL	5
ViaStain™ Live Cell Nuclear Red	CS1-V0009-1	5 mM	50 µL	5
ViaStain™ Live Cell Nuclear Far Red	CS1-V0010-1	5 mM	40 µL	5
ViaStain™ Live Cell Nuclear Blue	CS1-V0011-1	5 mM	100 µL	5

- Celigo Live Nuclear Dye Product Insert

1.3 Additional Equipment and Materials Required

- 15-mL tube
- Pipette and tips (P10, P200, P1000)
- Serological pipets and pipet aide (10mL)
- 96-well flat bottom, clear bottom, black walled
- Cells with appropriate media

1.4 Instrument and Software Requirements

- Celigo® Imaging Cytometer instrument

2.0 Assay Protocol

2.1 Preparation of Adherent Cells

- Adherent cells are plated at a cell density appropriate for well area coverage and length of drug treatment so cells are not over confluent at end of experiment. Take into account the number of cell divisions for the control well. At experiment end, the max cell number is between 30,000 – 40,000 cells/well. For example, plate 15000 cells (overnight adherence) in a 96-well format with 4 hour drug treatment. Verses, plate 5000 cells (overnight adherence) in 96-well format with 24-48 hour drug treatment.
- Adherent cells do not have to be trypsinized or pre-treated for this assay. Cell growth, staining and imaging may be done in the same plate.

2.2 Preparation of Suspension Cells

- Suspension cells are plated at a cell density appropriate for well area coverage and length of drug treatment with a total volume per well of 100 μ L.

2.3 Staining Procedure for a 96 well plate

For this example of a 96-well plate, prepare a 2X master mix using the following reagents: Diluent (cell culture media or PBS) and Live Cell Nuclear Dye. Some media may have background auto-fluorescence with the dyes, and therefore PBS is recommended in place of media.

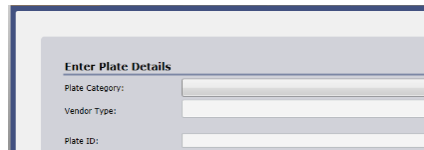
1. **2X Master Mix Preparation:**
 - a. Pipette 10 mL of diluent (cell media or PBS) into a 15-mL centrifuge tube.
 - b. Add appropriate volume of dye according to table below for each specific Live Nuclear Dye. Cap and invert to mix solution.
2. To wells containing 100 μ L with cells, pipette 100 μ L of 2X Master Mix, very gently into each well, so not to disturb lightly attached cells.
3. Incubate the plate for 15-30 min at (37°C) in the dark.
4. Plate is ready for imaging on Celigo. This an add-and-read assay, no washing required.

Product Name	Catalog No.	Stock	Add Stock volume to 10 mL PBS	[2X Master Mix]	Final [] (in well)
ViaStain™ Live Cell Nuclear Green	CS1-V0008-1	5 mM	20 μ L	10 μ M	5 μ M
ViaStain™ Live Cell Nuclear Red	CS1-V0009-1	5 mM	10 μ L	5 μ M	2.5 μ M
ViaStain™ Live Cell Nuclear Far Red	CS1-V0010-1	5 mM	8 μ L	4 μ M	2.0 μ M
ViaStain™ Live Cell Nuclear Blue	CS1-V0011-1	5 mM	20 μ L	10 μ M	5 μ M

3.0 Celigo Image Acquisition, Analysis, and Results

3.1 Home / Setup Tabs:

1. Select Create a New Scan
2. Select appropriate plate category and vendor type
3. Type in a Plate ID, or select an old one
4. Click Load Plate

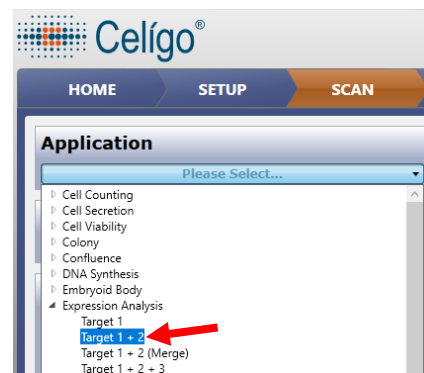


3.2 Scan Tab:

3.2.1 Target 1 Channel Setup

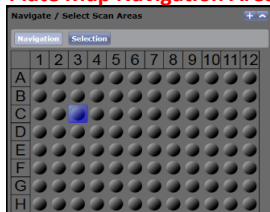
1. Select Application Expression Analysis: Target 1+2

Target 1 will be the fluorescent dye and Target 2 will be for a brightfield image. Customization of the channel names can be done with the customize button. See user Guide for details on how to customize channel names.

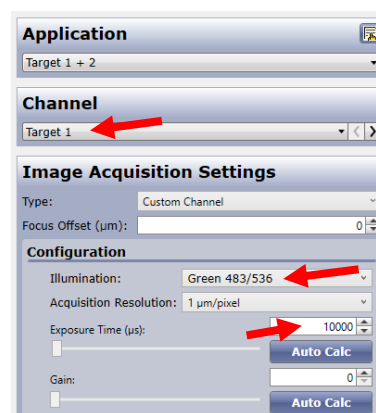


2. Select a well with sufficient number of Live Nuclear dye stained cells by clicking on plate map navigation area

Plate Map Navigation Area



3. Select Channel to Target 1
4. Set illumination to correspond to appropriate dye.
5. Set illumination exposure time for channel to appropriate exposure (μ s). See suggestions in table below.



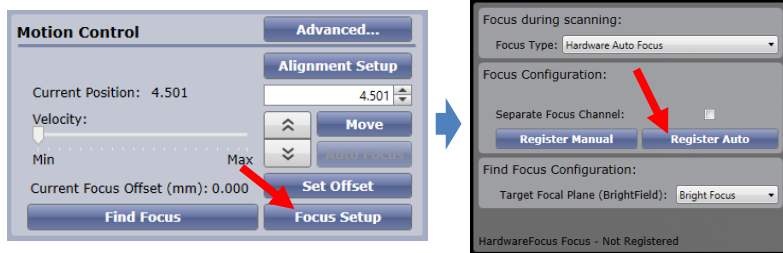
Product Name	Catalog No.	Celigo Illumination	Exposure (μ s)
ViaStain™ Live Cell Nuclear Green	CS1-V0008	Green 483/536	10,000
ViaStain™ Live Cell Nuclear Red	CS1-V0009	Red 531/629	10,000
ViaStain™ Live Cell Nuclear Far Red	CS1-V0010	Far Red 628/688	15,000
ViaStain™ Live Cell Nuclear Blue	CS1-V0011	Blue 377/470	75,000

- This exposure time should correspond to objects (nuclei) average pixel intensity values between 150-175. Adjust exposure accordingly, as the table is only a guide.

Hover Mouse over object to observe Pixel Intensity Display



- Click Focus Setup – Register Auto (with Hardware Auto Focus)

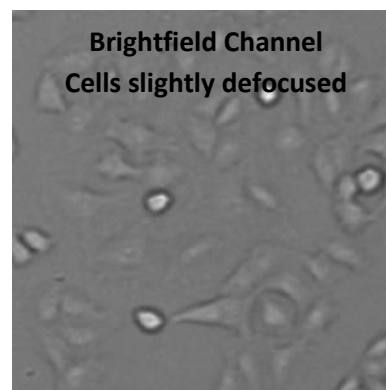
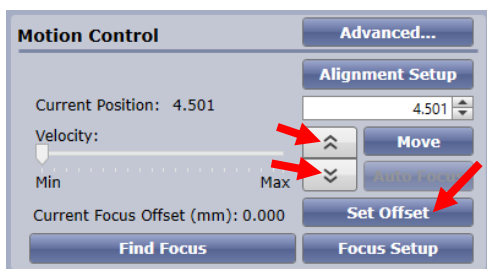
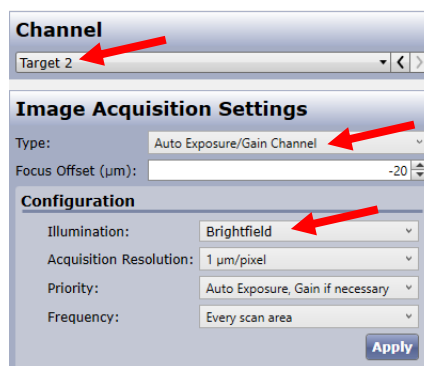


- Close dialog by clicking Focus Setup

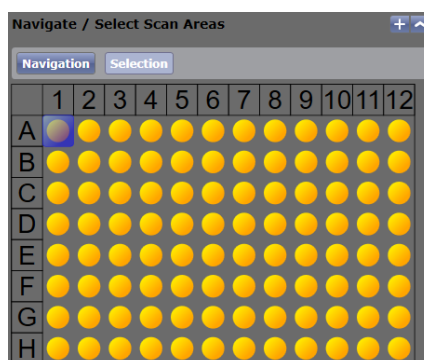
3.2.2 Target 2 Channel Setup

Target 1 will be the fluorescent dye and Target 2 will be for a brightfield image. Customization of the channel names can be done with the customize button. See user Guide for details on how to customize channel names.

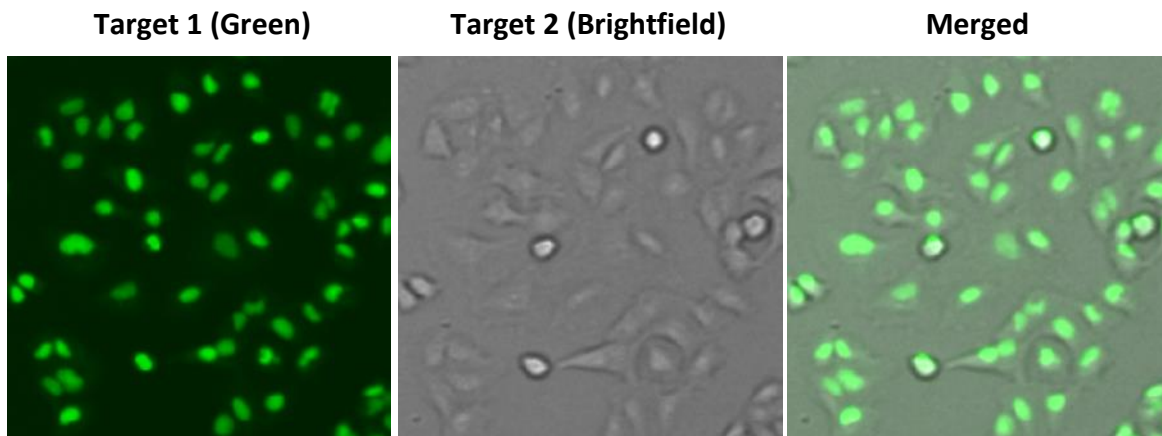
- Select Channel to Target 2
- Set illumination to Brightfield
- Set Type to Auto Exposure/Gain Channel
- Click Apply
- Adjust focus (double arrows) for cells to be slightly defocused
- Click Set Offset



- Click Selection on plate map navigation area and highlight wells for imaging
- Click Start Scan

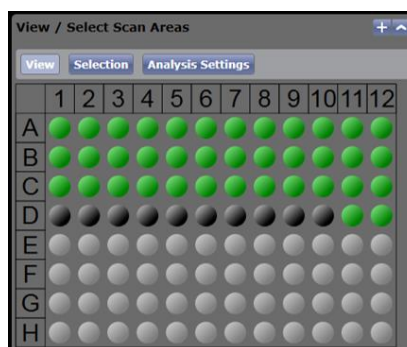


Example Images for Live Nuclear Green and Brightfield cells



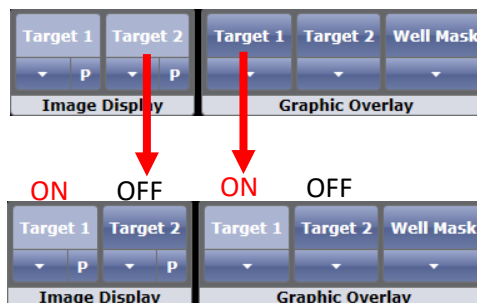
3.3 Analyze Tab:

1. Select a green well in plate map navigation area to view image in display area



3.3.1 "Target 1" Channel Analysis Setup

1. Turn ON Target 1 Graphic Overlay by clicking on word "Target 1"
2. Turn OFF Target 2 Image Display by clicking on word "Target 2"
 - Light blue indicates ON
 - Dark blue indicates OFF

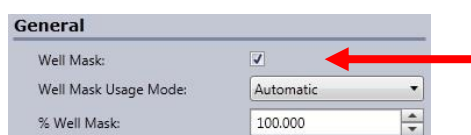


3. Click on an edge image in Well Map to view edge of well.

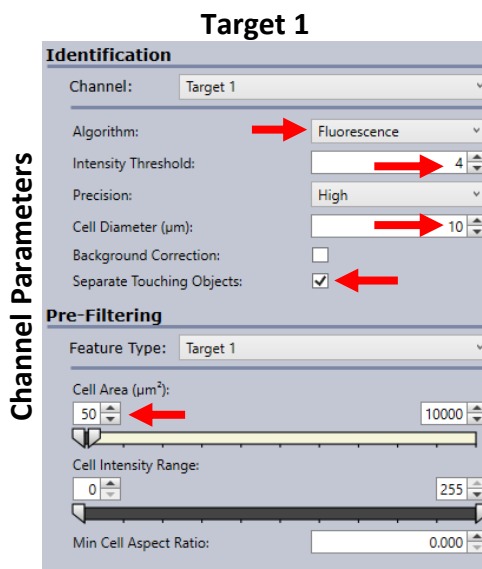
Well Map



Check box ON for Well Mask, to exclude object detection outside well area



4. In Identification Channel for Target 1, select Algorithm of Fluorescence (Default)
5. Adjust Intensity Threshold value to properly detect (outline) nuclei in image (Default 4)
 - Higher values detect only brighter objects
 - Lower values will include more dim objects
6. Adjust Cell Diameter (μm) to be average expected diameter of object (Default 10)
 - Higher values will expand current objects area
 - Lower values will decrease current objects area
7. Check box Separate Touching Objects to separate nuclei that are close together and identified as one object.
8. In Pre-filtering, increase lower limit of Cell Area to eliminate objects smaller than object of interest. Suggested minimum value of 50

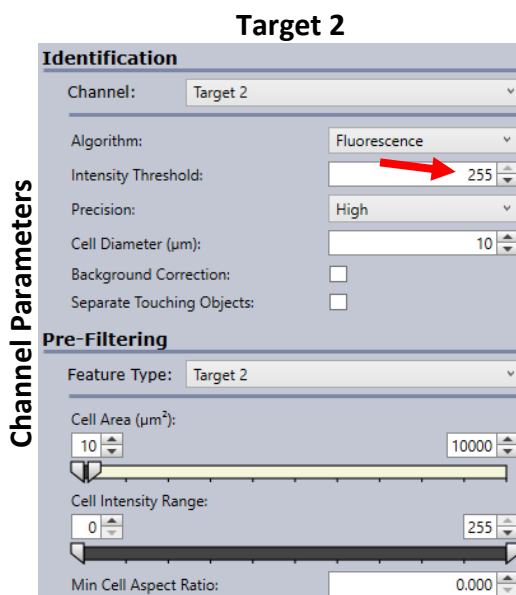


3.3.2 "Target 2" Channel Analysis Setup (Brightfield)

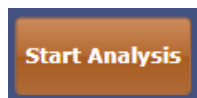
1. Turn ON Target 2 Image Display, turn OFF Target 2 Image Display so that you are only viewing the Target 2 Brightfield image
2. Turn ON Target 2 Graphic Overlay, turn OFF Target 1 Graphic Overlay so that you are only viewing Target 2 graphic overlay



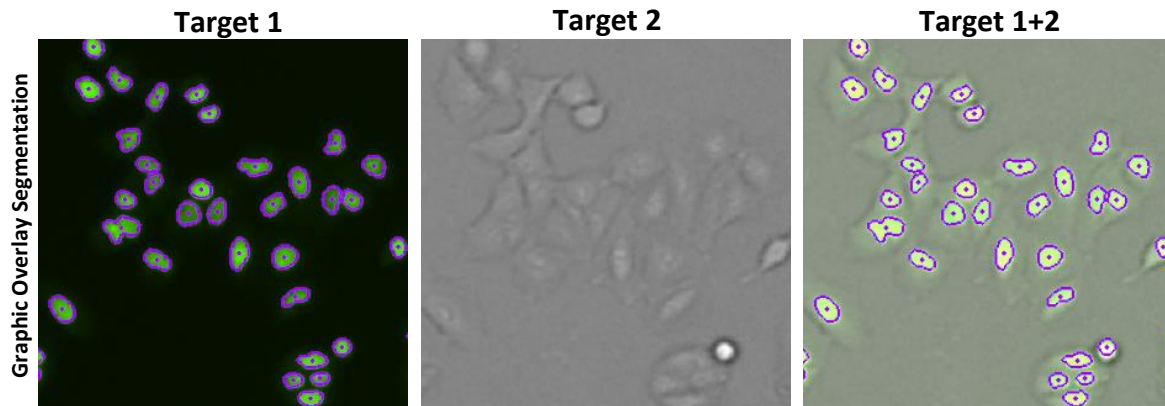
3. Change Identification channel to Target 2
4. Adjust Intensity Threshold to 255 to not detect any cells



5. Click Start Analysis



Example Images for Live Nuclear Green with Graphic Overlay Identification ON



3.4 Results Tab:

1. Once data analysis is finished, click Export Well Level Data into CSV file format.
 - Data reported are counts, mean and integrated fluorescent intensity measurements, and analysis parameters for current analysis. Disregard percentages.
2. Double click on a well to view whole image.
3. Scroll in to see high resolution images and graphic overlay



4.0 Reference

4.1 Quick Reference Recommended Values

Image plate at the suggested following exposures to obtain object pixel intensities between 150-175:

- Live Dye– Target 1 Channel see table below, Focus Setup with HWAF or IBAF
- BF – Target 2 Channel @ Auto Exposure/Gain Channel, Focus offset value

Product Name	Catalog No.	Celigo Illumination	Exposure (µsec)
ViaStain™ Live Cell Nuclear Green	CS1-V0008	Green 483/536	10,000
ViaStain™ Live Cell Nuclear Red	CS1-V0009	Red 531/629	10,000
ViaStain™ Live Cell Nuclear Far Red	CS1-V0010	Far Red 628/688	15,000
ViaStain™ Live Cell Nuclear Blue	CS1-V0011	Blue 377/470	75,000

4.2 Technical Support

- Celigo Learning Center (online) at www.nexcelom.com/celigo-learning
- Nexcelom Technical Support is available from 9am to 5:00pm EST.

E-mail: support@nexcelom.com

Phone: 978-327-5340

4.3 Storage and Handling

- For long term storage, store between -16°C to -24°C.
- Thaw to room temperature before use.

4.4 Warranty

This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use. Product is warranted to meet the specifications outlined in the Certificate of Analysis when stored and used according to the manufacturer's instructions. No other warranty, expressed or implied (such as merchantability, fitness for a particular purpose, or non-infringement) is granted. Warranty is valid until the expiration date stated on the product label. If no expiration is listed, the warranty is valid for 6 months from the date of product receipt. Warranty will be void if product is stored incorrectly, the recommended protocol is not followed, or the product is used for a different application.

5.0 Ordering Information

5.1 How to Reorder

For orders shipping to destinations in the United States:

- When ordering with a Purchase order
 - Fax a copy of your order to 978-327-5341
 - Email a copy of your order to sales@nexcelom.com
- When ordering with a Credit Card
 - Visit www.shop.nexcelom.com and place your order

For orders shipping to destinations outside the United States:

Contact your local distributor or Nexcelom Representative to place your order