



ViaStain™ Hoechst/PI Viability Kit

Product Number: CSK-V0005-1

Sample Kit: CSK-V0005-S



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

Table of Contents

1. Introduction

1.1 Description of Assay.....	Page 2
1.2 Materials Included.....	Page 2
1.3 Additional Equipment and Materials Required.....	Page 2
1.4 Instrument and Software Requirements.....	Page 2

2. Assay Protocol

2.1 Preparation of Adherent Cells.....	Page 3
2.2 Preparation of Suspension Cells.....	Page 3
2.3 Preparation of Positive and Negative Control Samples.....	Page 3
2.4 Staining Procedure for a 96 well plate.....	Page 3

3. Celigo Protocol (Image Acquisition, Analysis, Results)

3.1 Start Tab.....	Page 4
3.2 Scan Tab.....	Page 4
3.3 Analysis Tab.....	Page 5
3.4 Results Tab.....	Page 7

4. Reference

4.1 Quick Reference Recommended Values.....	Page 8
4.2 Citations	Page 8
4.3 Technical Support.....	Page 8
4.4 Storage and Handling.....	Page 8

5. Ordering Information

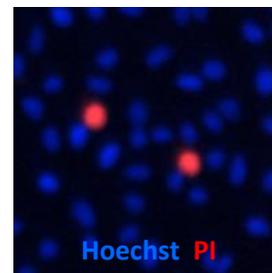
5.1 How to Reorder.....	Page 9
-------------------------	--------

1.0 Introduction

1.1 Description of Assay

Viability Assay:

In this viability assay, the total number of nucleated cells and the total number of dead cells are identified by staining the cells with Hoechst and Propidium Iodide (PI). Propidium Iodide is membrane impermeable and only enters the dead (membrane compromised) cells, where upon entry it binds to DNA in an intercalating manner. Hoechst 33342 is a DNA binding agent that is membrane permeable and is used to identify all nucleated cells. After staining the cells with Hoechst/PI, the Celigo instrument can scan, analyze and report the number and percent of live and dead cells on a per well basis.



1.2 Materials and Reagents

- ViaStain™ Hoechst/PI
Cat. # CSK-V0005-1 (Stains 5, 96 well plates)
 - (component A) Propidium Iodide, 250 µL
 - (component B) Hoechst 33342, 50 µL
- ViaStain™ Hoechst/PI – Sample kit (not available for individual re-sale)
Cat. # CSK-V0005-S (Stains 1, 96 well plates)
 - (component A) Propidium iodide, 50 µL
 - (component B) Hoechst 33342, 10 µL
- ViaStain™ Hoechst/PI Kit Instructions

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, so at experiment end the max cell number is between 20,000 – 25,000 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 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.

2.3 Preparation of Positive and Negative Control Samples

- A positive and negative control well should be used to check exposure time and optimize analysis intensity threshold for dead and total cells.
- A positive control may be generated by exposing cells to a death inducing pharmacological agents such as α -TOS, Etoposide, or Staurosporine. For Celigo experiments, A549 cells were treated with 50 μ M α -TOS or 200 nM Staurosporine for 24 hours.
- A negative control (untreated cells) should be tested to determine baseline cell count and percent viability of cells.
- Positive and negative controls should be processed at the same time using the staining and data acquisition procedures outlined below.

2.4 Staining Procedure for a 96 well plate

For a 96-well plate prepare a 4X master mix using the following reagents: 1x PBS (or cell media), Propidium iodide (PI) and Hoechst 33342.

1. Prepare a 4X Master Mix staining solution:
 - a. Pipette 5 mL of cell culture media or 1x PBS into a 15 mL centrifuge tube.
 - b. Add 40 μ l of the Propidium iodide (Component A) to the 15 mL tube.
 - c. Add 8 μ l of the Hoechst 33342 (Component B) to the 15 mL tube.
 - d. Vortex tube for 10 seconds.
2. Slowly pipette 50 μ l of 4X Master Mix staining solution into each well containing 150 μ l of media, do not disturb the lightly attached dead cells. Final volume per well will now be 200 μ l.
3. Incubate the plate for 15-30 min at RT (25°C) in the dark.
4. This an add and read assay, no washing required.
5. Plate is ready for imaging on Celigo.

3.0 Celigo Image Acquisition, Analysis, and Results

3.1 Start Tab:

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

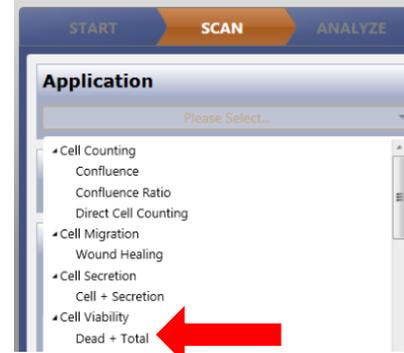
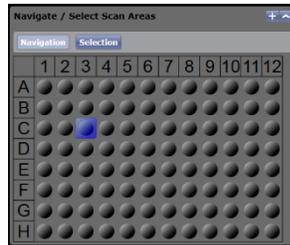


3.2 Scan Tab:

3.2.1 Total Channel Setup

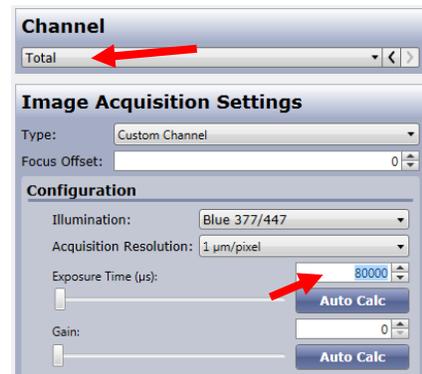
1. Select Application Cell Viability: Dead + Total.
2. Select a well with sufficient number of PI (dead) and Hoechst stained cells by clicking on plate map navigation area.

Plate Map
Navigation
Area

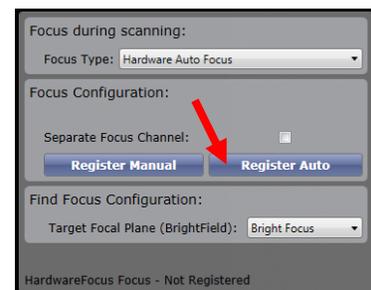


3. Change Channel to Total.
4. Set illumination exposure time for blue channel to 80,000 μs.
 - This exposure time should correspond to objects (nuclei) average pixel intensity values between 150-175. Adjust exposure accordingly.

Hover Mouse
over object to
observe Pixel
Intensity Display



5. Click Focus Setup – Register Auto (with Hardware Auto Focus) – close dialog by clicking Focus Setup.



3.2.2 Dead Channel Setup

1. Change channel to Dead.
2. Change illumination to Red Fluorescence.
3. Set illumination exposure time for red channel to 20,000 -40,000 μ s.
 - This exposure time should correspond to objects (dead cells) average pixel intensity values between 150-175. Adjust exposure accordingly.

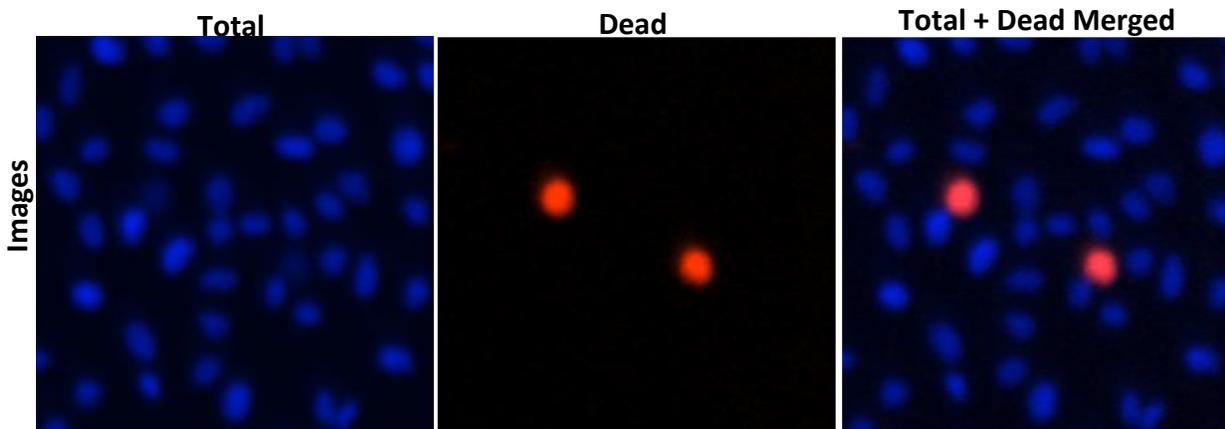
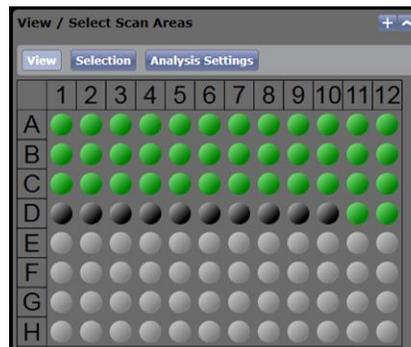


4. Click Find Focus to let the system find the correct focal plane.
5. Click Set Offset once correct focal plane was found.
6. Click Selection on plate map navigation area and highlight wells for imaging.
7. Click Start Scan.



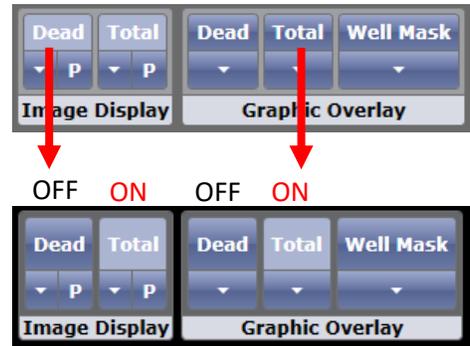
3.3 Analyze Tab:

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

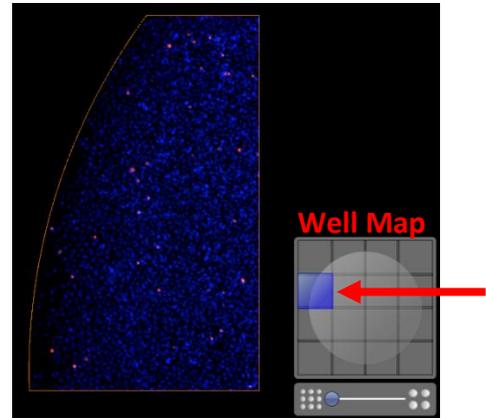
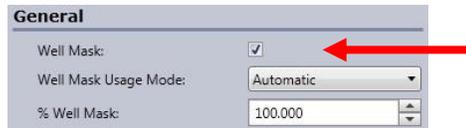


3.3.1 “Total Channel” Analysis Setup

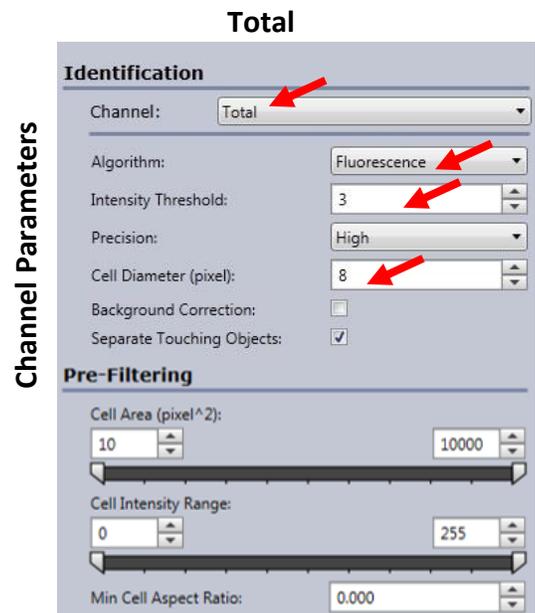
1. Turn ON Total Graphic Overlay by clicking on word “Total”.
2. Turn OFF Dead Image Display by clicking on word “Dead”.
 - Light blue indicates ON
 - Dark blue indicates OFF



3. Click on an edge image in Well Map to view edge of well.
4. Check box ON for Well Mask, to exclude object detection outside well area.



5. Change Identification Channel to Total.
6. Select Algorithm of Fluorescence (Default).
7. Adjust Intensity Threshold value to properly detect (outline) nuclei in image.
 - Higher values detect only brighter objects.
 - Lower values will include more dim objects.
8. Adjust Cell Diameter (um) to be average expected diameter of object.
 - Higher values will expand current objects area.
 - Lower values will decrease current objects area.
9. Check box Separate Touching Objects to separate nuclei that are close together and identified as one object.
10. In Pre-filtering, increase lower limit of Cell Area to eliminate objects smaller than object of interest. Suggested minimum value of 30.

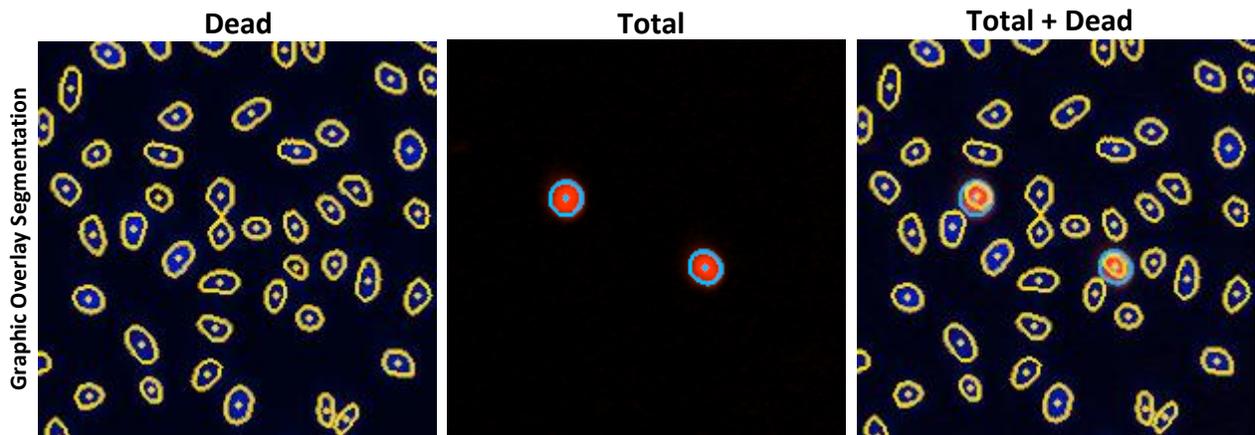
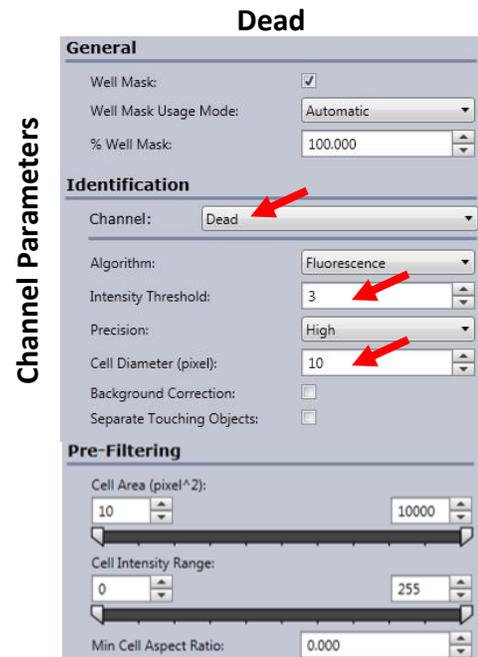


3.3.2 “Dead Channel” Analysis Setup

1. Turn ON Dead Image Display, turn OFF Total Image Display so that you are only viewing the Dead image.
2. Turn On Dead Graphic Overlay, turn OFF Total Graphic Overlay so that you are only viewing Dead graphic overlay.
3. Change Identification channel to Dead.
4. Adjust Intensity Threshold and Cell Diameter to properly detect Dead Cells.
5. Adjust Pre-filtering values as needed.



6. Click Start Analysis.



3.4 Results Tab:

1. Once data analysis is finished, click Export Well Level Data into CSV file format.
 - Data reported are % Dead, Dead Cell counts, Total Cell counts, mean and integrated fluorescent intensity measurements, and analysis parameters for current analysis.
2. Double click on a wells to view whole.
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:

- Hoechst – Total Channel @ 80,000 μ s, Focus Setup with HWAF or IBAF.
- PI – Dead Channel @ 40,000 μ s, Focus offset value of -20 μ m.

4.2 Citations

1. Chen HY, et al. (2013). Inhibition of redox/Fyn/c-Cbl pathway function by Cdc42 controls tumor initiation capacity and tamoxifen sensitivity in basal-like breast cancer cells. *EMBO Mol. Med* 5(5): 723-736.

4.3 Technical Support

- Celigo Learning Center (online) at www.nexcelom.com/celigo-learning
- Nexcelom Technical Support is available from 9am to 5:30pm EST.
E-mail: support@nexcelom.com
Phone: 978-327-5340

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 12 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.

4.5 Storage and Handling

- For long term storage, store ViaStain™ Hoechst 33342 (Component B) between -16°C to -24°C. Thaw to room temperature before use.
- For long term storage, store ViaStain™ Propidium iodide (Component A) between 2°C to 8°C.

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