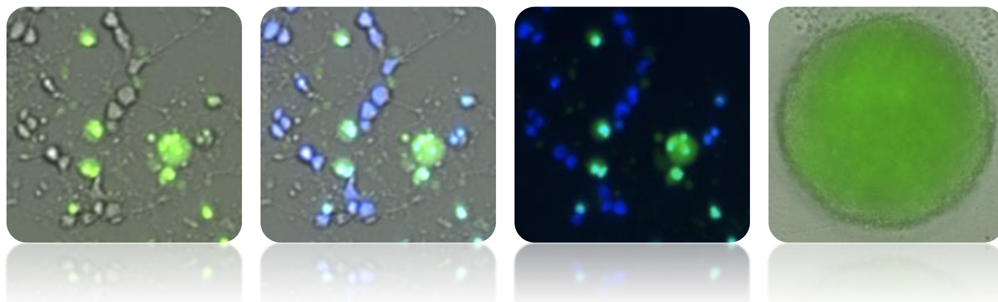
ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture

Product Numbers: CS1-V0002-1, CSK-V0003-1



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

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1.0 Introduction

1.1 Assay Description

Nexcelom's ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture kit is designed to detect caspase 3/7 activity in live cells. The ability to perform kinetic apoptosis assays allows researchers to continuously measure the caspase 3/7 activity within the cell population. This no wash assay has been shown to effectively work in both 2D and 3D cultures.

The reagent (NucView™) consists of a nucleic acid-binding dye with a fluorescent probe that is attached to a four-amino acid peptide sequence DEVD (Asp-Glu-Val-Asp) forming a cell membrane-permeable DEVD-DNA complex. While the nucleic-acid dye is linked to the DEVD peptide sequence, the dye is unable to bind to DNA and remains non-fluorescent. During apoptosis, caspase 3/7 proteins cleave the DEVD-DNA dye complex and thereby release the high-affinity DNA dye, which translocates to the nucleus and binds to the DNA, producing a bright green fluorescent signal.

1.2 Materials Included

- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture
  Cat. # CS1-V0002-1 (Stains ~ 2.5 96-well plates)
    - Caspase 3/7 reagent at 1 mM in DMSO.

Or

- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture with Hoechst 33342
  Cat. # CSK-V0003-1 (Stains ~ 2.5 96-well plates)
    - Caspase 3/7 reagent at 1 mM in DMSO
    - Hoechst 33342 at 20 mM in water
- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture Kit instructions

1.3 Additional Materials Required, Not Included

- 15-mL tube
- Pipette and tips (P10, P200, P1000)
- Serological pipettes and pipette aide (10 mL)
- 96-well flat bottom, clear bottom plate *(Recommended: Greiner #655090)*
- Cells with appropriate media

1.4 Instrument and Software

- Celigo® S Image Cytometer or equivalent fluorescent imaging platform

2.0 Assay Protocol for 2D Caspase 3/7 Detection

2.1 Preparation of Control Samples

- A positive control may be generated by exposing cells to a pharmacological agent. For example, MDA-MB-231 cells were treated with 3 µM Staurosporine for a period of 5-7 hours.
- A negative control (untreated cells) should be tested to determine the baseline caspase 3/7 activity of the cells.

2.2 Staining Procedure for Continuous Monitoring of Caspase 3/7 Activity

- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture should be used at a final concentration between 2 – 5 µM. For Celigo applications, we recommend a starting concentration of 2 µM.

2.2.1 Adherent Cell Procedure:

1. For adherent cells in a 96-well plate, seed approximately 5,000-10,000 adherent cells per well and allow the adherent cells to attach overnight.
    a. Prepare in 10 mL media, a cell concentration of $5x10^4$ adherent cells/mL.
    b. Pipet 100 µL per well in 96-well plate.
    c. Incubate overnight at 37°C.
2. Prepare a 2X concentration (4 µM) of caspase 3/7 substrate in media.
    a. In 10 mL media, add 40 µL of 1mM caspase 3/7 substrate.
3. Prepare a 2X concentration drug treatment.
4. Remove all media from wells with adherent cells.
5. Add 100 µL of the 2X caspase 3/7 to all wells.
6. Add 100 µL of 2X drug treatment to wells.
    - Combining the 2X solutions from steps 5 & 6 in the well, it will become a 1X caspase 3/7 and 1X drug treatment final in wells.
7. Incubate at 37°C for the experiment span of the continuous monitoring.
8. Image at multiple time points during the drug treatment on Celigo or equivalent fluorescent imaging platform.
    - Optional: Hoechst can be added at 8 µM final at the end of continuous monitoring for total nuclear staining. Remove 100 µL from wells, then add 100 µL 2X (16 µM) Hoechst to wells. Incubate for 30 minutes at 37°C. For a full protocol using Hoechst in an end-point assay, see section 2.3.

2.2.2 Suspension Cell Procedure:

- For suspension cells, plate cells with caspase 3/7 substrate on day of experimental drug treatment. (10,000-20,000 cells per well). No overnight incubation is necessary.

1. Prepare in 10 mL media, suspension cells at $1\text{-}2x10^5$ cells/mL concentration.
2. Add 40 µL of caspase 3/7 substrate to cell suspension, which makes a 4 µM (2X) concentration.
3. Prepare 2X concentration drug treatments.
4. Pipet 100 µL/well of suspension cell + caspase 3/7 substrate mix to 96-well plate.
5. Pipet 100 µL of 2X drug treatment to wells.

- Combining the 2X solutions from steps 4 & 5 in the well, it will become a 1X caspase 3/7 and 1X drug treatment final in wells.

6. Centrifuge plate (500 rpm quick up/down) to settle cells.
7. Incubate at 37°C for the experiment span of the continuous monitoring.
8. Image at multiple time points during the drug treatment on Celigo or equivalent fluorescent imaging platform.
    - Optional: Hoechst can be added at 8 µM final at the end of continuous monitoring for total nuclear staining. Remove 100 µL from wells, then add 100 µL 2X (16 µM) Hoechst to wells. Incubate for 30 minutes at 37°C. For a full protocol using Hoechst in an end-point assay, see section 2.3.

2.3 Staining Procedure for Endpoint Assay with Hoechst

- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture should be used at a final concentration between 2 – 5 µM. For Celigo applications, we recommend a starting concentration of 2 µM.
- Hoechst 33342 for total nuclear detection should be used at a final concentration between 5-15 µM. For Celigo applications, we recommend a starting concentration of 8 µM.

2.3.1 Adherent Cell Procedure:

1. In a 96-well plate, seed approximately 5,000-10,000 adherent cells per well and allow the adherent cells to attach overnight at 37°C.
    a. Prepare in 10 mL media, a cell concentration of $5x10^4$ adherent cells/mL.
    b. Pipet 100 µL per well in 96-well plate.
    c. Incubate overnight at 37°C.
2. Drug treat your cells for a predetermined time.
3. Prepare a 2 µM caspase 3/7 and 8 µM Hoechst 33342 concentration master mix in PBS (once mixed with PBS the solution must be used within 3 hours).
    a. Into 20 mL of PBS, add 40 µL of caspase 3/7 substrate and 8 µL of Hoechst 33342.
4. Post desired drug treatment, aspirate all media from wells.
5. Pipet 200 µL of the master mix into each well.
6. Incubate for 30 minutes at 37°C.
7. Image on Celigo or equivalent fluorescent imaging platform.

2.3.2 Suspension Cell Procedure:

- For suspension cells, plate cells on the day of the experiment. No overnight incubation is necessary.

1. In a 96-well plate, seed approximately 10,000-20,000 suspension cells per well.
    a. Prepare in 10 mL media a cell concentration of $1\text{-}2x10^5$ cells/mL.
    b. Pipet 100 µL/well of cell to 96-well plate.
2. Prepare 2X concentration drug treatments.
3. Pipet 100 µL of 2X drug treatment to wells.

- Combining the 2X solution from steps 1b & 3 in the well, it will become a 1X drug treatment final in wells.

4. Incubate at 37°C for appropriate time for drug treatment to have effect.
5. Prepare a 2X Master mix of 4 µM caspase 3/7 substrate and 16 µM Hoechst 33342.
    a. In 10 mL of PBS, add 40 µL caspase 3/7 substrate and 8 µL Hoechst 33342.
6. Centrifuge plate (500 rpm quick up/down) to settle cells.
7. Remove 100 µL from each well, careful not to disturb cells at well bottom.
8. Pipet 100 µL of 2X master staining mix to each well.
9. Incubate for 30 minutes at 37°C.
10. Image on Celigo or equivalent fluorescent imaging platform.
    - If cells have clumped together or rolled to well edge, pipet up/down to resuspend cells in well. Quick centrifuge (500 rpm up/down) to settle cells evenly on bottom.

3. Assay Protocol for 3D of Caspase 3/7 Detection

3.1 Preparation of Control Samples

- A positive control may be generated by exposing spheres to a pharmacological agent.
- A negative control (untreated spheres) should be tested to determine the baseline caspase 3/7 activity of the cells.

3.2 Preparation of Multicellular Tumor Spheroids (MCTSs)

- Prepare MCTSs in either flat bottom or U-bottom plates 96 well plates

3.3 Staining Procedure for Continuous Monitoring of Caspase 3/7 Activity

- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture should be used at a final concentration between 2 – 5 µM. For Celigo applications, we recommend a starting concentration of 2 µM.
- Volumes for assay are for 96well plate; adjust for 384-well plate.

1. Prepare a 4X concentration of caspase 3/7 substrate (8 µM) in media.
    a. In 5 mL of PBS, add 40 µL caspase 3/7 substrate
2. Prepare a 4X concentration drug treatment.
3. Adjust well volume so that each wells contains 100 µL media.
4. Add 50 µL of the 4X caspase 3/7 to all wells.
5. Add 50 µL of 4X drug treatment to wells.
    - By adding the 4X solutions from steps 4 & 5 into the wells containing 100 µL of media results in a final 1X caspase 3/7 (2 µM) and 1X drug treatment in each well.
6. Incubate for 60 minutes at 37°C.
7. Image at multiple time points during the drug treatment on Celigo or equivalent fluorescent imaging platform.

3.4 Staining Procedure for End-point Assay with Hoechst

- ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture should be used at a final concentration between 2 – 5 µM. For Celigo applications, we recommend a starting concentration of 2 µM.
- Hoechst 33342 for total nuclear detection should be used at a final concentration between 5-15 µM. For Celigo applications, we recommend a starting concentration of 8 µM.
- Volumes for assay are for 96 well plate, adjust for 384-well plate.

1. Prepare a 2X concentration (4 µM caspase 3/7 and 16 µM Hoechst 33342) master mix of caspase substrate with Hoechst 33342.
   a. In 10 mL of PBS, add 40 µL caspase 3/7 substrate and 8 µL Hoechst 33342
2. Using a 96-well plate containing 100 µL of volume per well, add 100 µL of the 2X master mix.
3. Incubate for 60 minutes at 37°C.
4. Image on Celigo or equivalent fluorescent imaging platform.

4. Celigo Instrument Parameters for 2D Continuous Monitoring Caspase 3/7 Detection

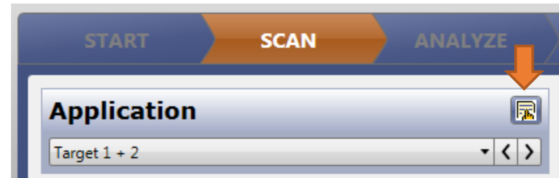
4.1 Start Tab:

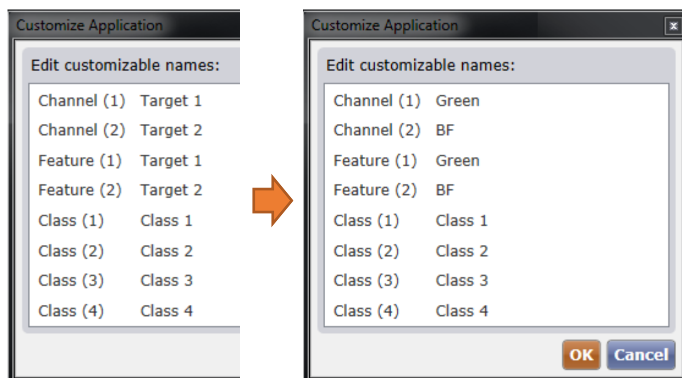
1. Select Create a New Scan.
2. Select appropriate Plate Category and Vendor Type.
3. Type in a Plate ID, or select an old one.
4. Click Load Plate.

4.2 Scan Tab:

4.2.1 Select an application

1. Select Application Expression Analysis Target 1+2.
2. Customize the channels with appropriate names by clicking on the "Customize Analysis Application" button.
   a) Change channel and feature names to the following: Target 1 (Green), Target 2 (Bright Field).
   b) Click OK.





4.2.2 Setup "Bright field" Channel

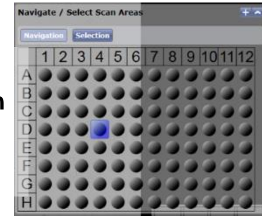
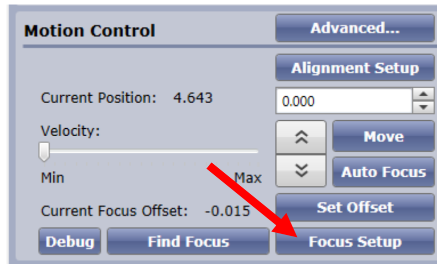
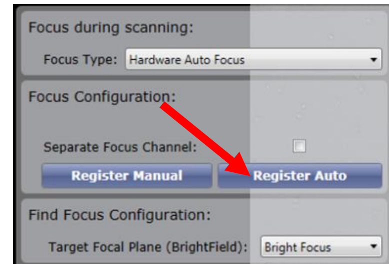


Plate Map Navigation Area

1. Select a well with sufficient number of caspase 3/7+ (such as a treated well) cells by clicking on plate map navigation area. Also, avoid using the outer wells on plate.
2. Change Channel to BF.
3. Change the illumination to Bright field.
4. Click AutoCalc to find the correct illumination to have background pixels between 120-150 relative FL units (RFUs).
5. Click Focus Setup.
6. Register Auto (with Hardware Auto Focus).
7. Close dialog by clicking Focus Setup.

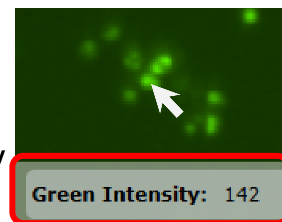


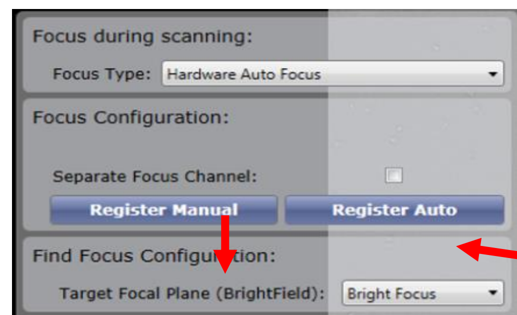


4.2.3 "Green" Channel Setup

1. Change Channel to Green.
2. Change illumination to Green fluorescence.
3. Set illumination exposure time for green channel to 50,000 µs.
   - This exposure time should correspond to objects (apoptotic cells) average pixel intensity values between 100-150 RFUs. We recommend 50,000-80,000 µs.
4. Click Find Focus to let the system find the correct focal plane. Alternatively, manually adjust z-focus with arrows to find the correct focus.
5. Click Set Offset once correct focal plane is found.
6. Click Selection on plate map navigation area and highlight wells for imaging.
7. Click Start Scan.

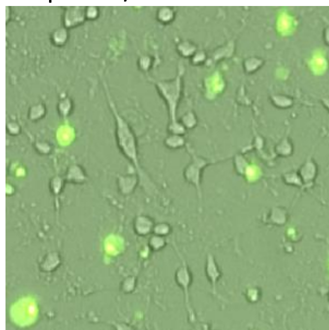
Hover Mouse over object to observe Pixel Intensity Display



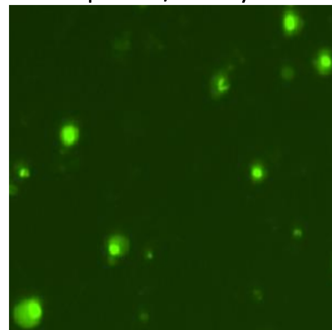


Images of different channel combinations:

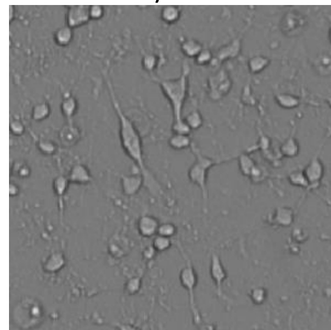
Caspase 3/7 + BF



Caspase 3/7 Only

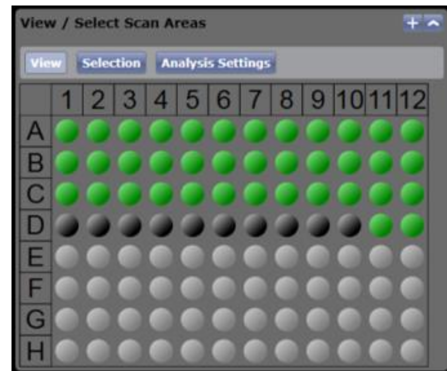


BF Only



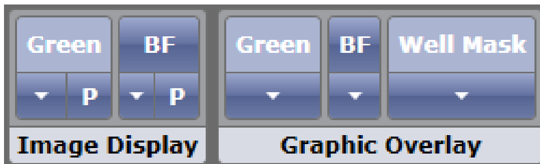
4.3 Analyze Tab:

4.3.2 "Green Channel" Analysis Setup

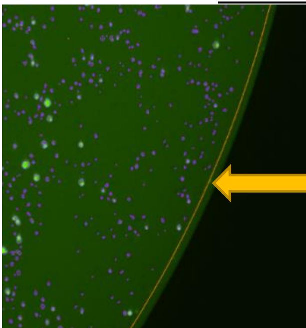


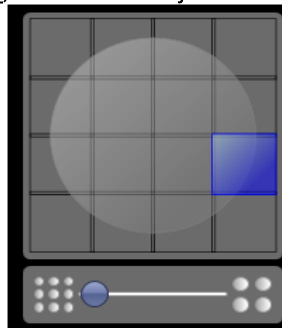
- As images are acquired for each well, the plate navigation map will show green wells for images saved to database.

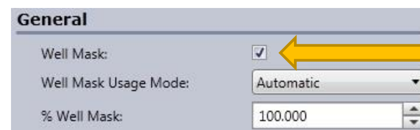
1. Select a green well in plate map navigation area to view image in display area.
2. Turn On Green Graphic Overlay, turn OFF BF Graphic Overlay so that you are only viewing green graphic overlay.
   - Light blue indicates ON
   - Dark blue indicates OFF

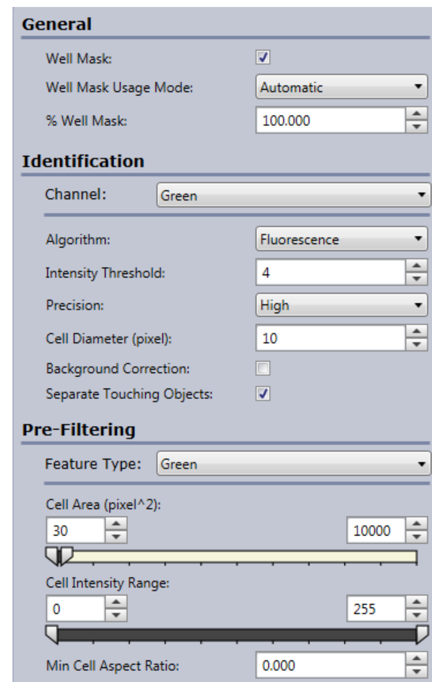


3. Click on an edge image in Well Map to view edge of well.
4. Check box ON for Well Mask, to exclude object detection outside well area.









5. Change Identification Channel to Green.
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 (µm) to be the 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 objects 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 values of 30-50 Cell Area (pixel^2).

4.3.1 "BF" Analysis Setup

1. Change the identification channel to BF.
2. Turn ON BF Graphic Overlay, turn OFF Green Graphic Overlay so that you are only viewing BF graphic overlay.
3. Select Algorithm Bright field.
4. Adjust Intensity Threshold and Cell Diameter to properly detect BF Cells.
5. Adjust Pre-filtering values as needed.
6. Once the analysis parameters for Green and BF are optimized, click Start Analysis.

4.4 Results Tab:

1. Double click on a well in the results page to view the whole-well image.
2. Scroll in to see high resolution images and graphic overlay.
3. Once data analysis is finished, click Export Well Level Data into CSV file format.

4. Data reported are Counts, Standard Deviation, Average Mean Intensities, Integrated Intensity measurements of all channels, and analysis parameters for current analysis.

5. Celigo Instrument Parameters for 2D Caspase 3/7 Endpoint Detection

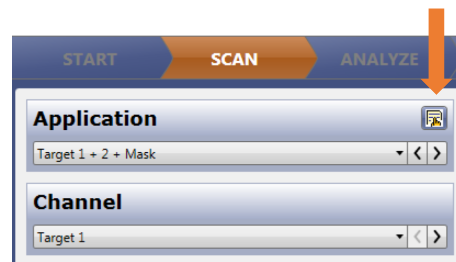
5.1 Start Tab:

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.

5.2 Scan Tab:

5.2.1 Select an application

1. Select Application Expression Analysis Target 1+2+Mask.
2. Customize the channels with appropriate names by clicking on the "Customize Analysis Application" button (Shown in the picture).
   c) Change channel and feature names to the following: Target 1 (Green), Target 2(Bright Field) and Mask (Blue).
   d) Change Class 1 to Caspase 3/7+.



e) Click OK.

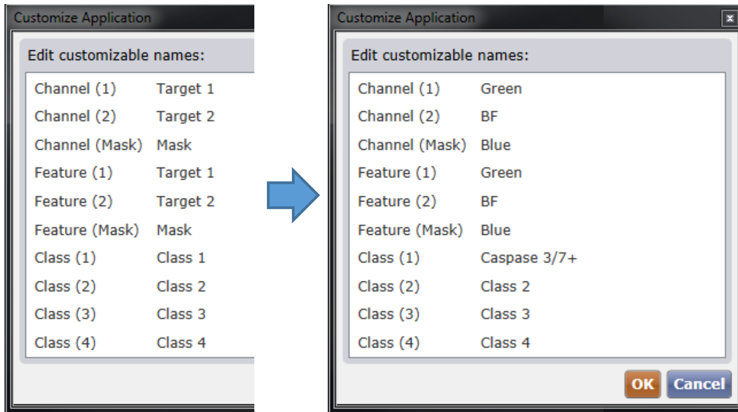
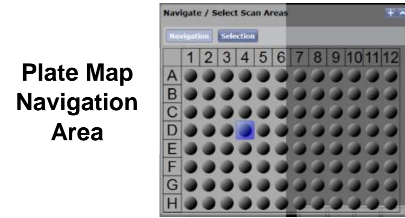


Plate Map Navigation Area

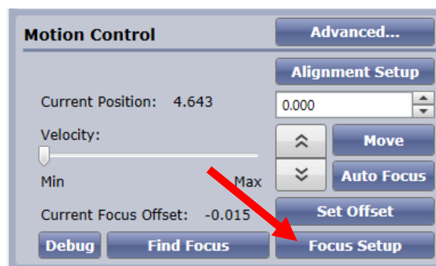


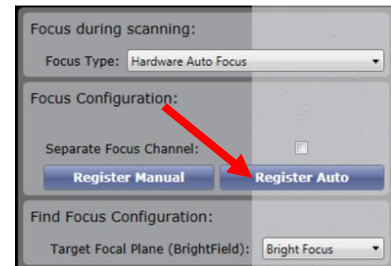
5.2.2 Setup Blue (Mask) Channel

1. Select a well with sufficient number of caspase 3/7+ and Hoechst stained cells by clicking on plate map navigation area.
2. Change Channel to Blue (Mask).
3. Set illumination exposure time for blue channel to 80,000 µs.
   - This exposure time should correspond to objects (nuclei) average pixel intensity values between 100-150 relative FL units.

Hover Mouse over object to observe Pixel Intensity Display

4. Click Focus Setup.
5. Register Auto (with Hardware Auto Focus).
6. Close dialog by clicking Focus Setup.





5.2.3 Green Channel Setup

1. Change channel to Green.
2. Change illumination to Green fluorescence.
3. Set illumination exposure time for green channel to 50,000 µs.
   - This exposure time should correspond to objects (apoptotic cells) average pixel intensity values between 100-150 relative FL units. We recommend 50,000-80,000 µs.
4. Click Find Focus to let the system find the correct focal plane or adjust manually with arrows.

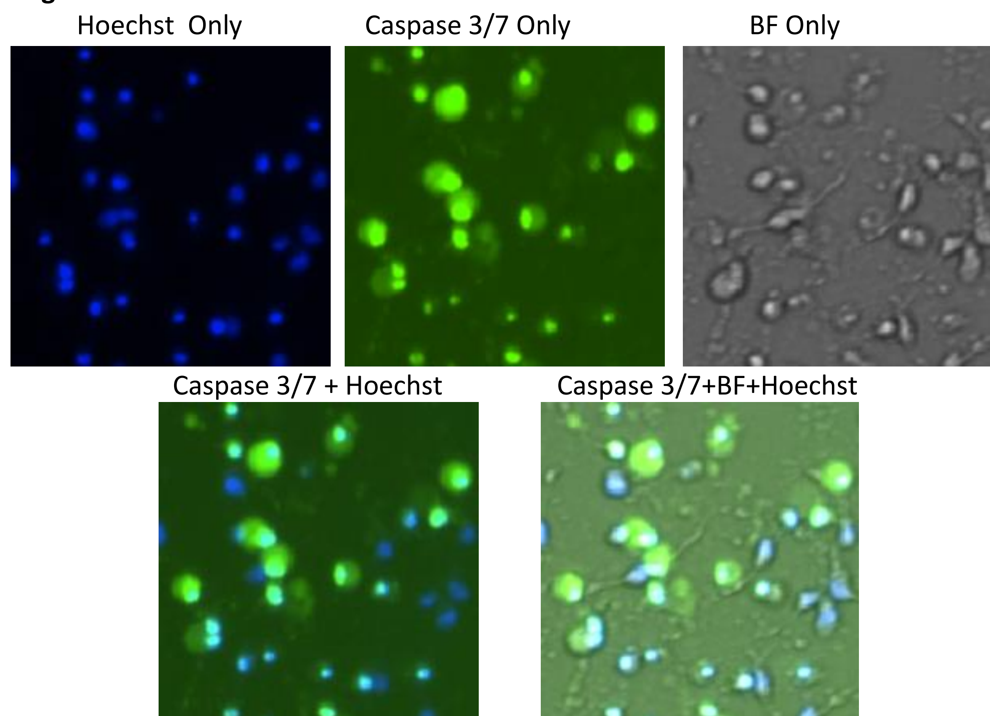
5. Click Set Offset once correct focal plane was found.

Note: Caspase 3/7+ substrate will photo bleach from frequent or long exposure of camera on to the cells. We recommend using Snap when possible versus Live camera.

5.2.4 Bright field Channel Setup

1. Change channel to BF.
2. Change the illumination to Bright field.
3. Click AutoCalc to find the correct illumination to have background pixels between 120 – 150.
4. Click Find Focus to let the system find the correct focal plane for BF.
5. Click Set Offset once the correct focal plane was found.
6. Click Selection on plate map navigation area and highlight wells for imaging.
7. Click Start Scan.

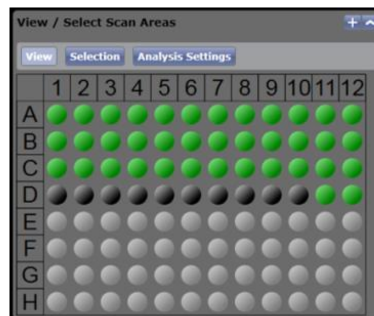
Example Images of Channels:



5.3 Analyze Tab:

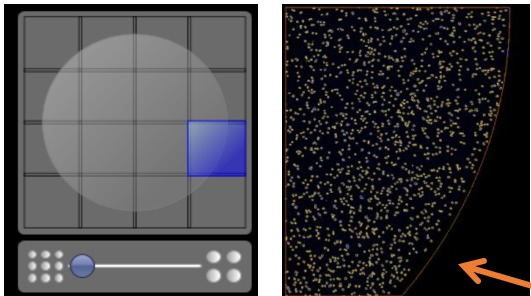
5.3.1 "Blue" Mask Channel Analysis Setup

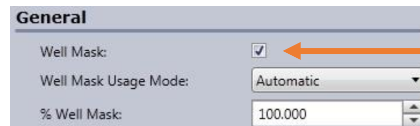
- The "Mask" channel is a single graphic overlay mask used to identify objects in one channel, typically the last channel. The Mask channel is used as a Total cell or object count. Additional channels will use the mask channel graphic overlay to measure fluorescent and bright field intensity for each object. Therefore, analysis parameters for the mask channel are the only ones needing to be optimized in Analyze Tab.
- Sub-populations are defined in the Gating Tab.
- As images are acquired for each well, the plate navigation map will show green wells for images saved to database.



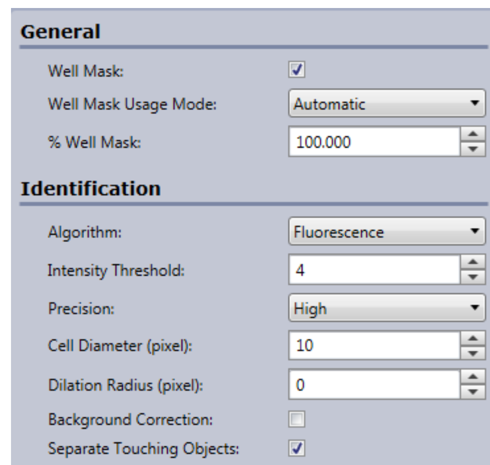
1. Select a green well in plate map navigation area to view image in display area.
2. Turn ON Blue Graphic Overlay, turn OFF Green and BF Graphic Overlay so that you are only viewing "Blue" graphic overlay.
    - Light blue indicates ON
    - Dark blue indicates OFF

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.





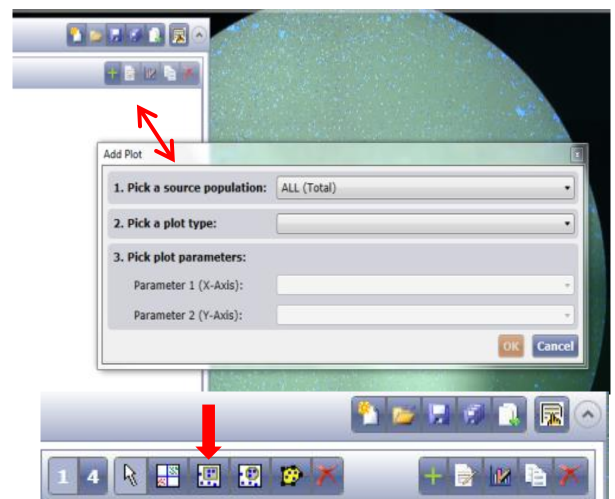
5. Select Algorithm of Fluorescence (Default).
6. Adjust Intensity Threshold value to properly detect (outline) nuclei in image.
    - Higher values detect only brighter objects
    - Lower values will include more dim objects
7. Adjust Cell Diameter (µm) to be average expected diameter of object.
    - Higher values will expand current objects area
    - Lower values will decrease current objects area
8. Check box Separate Touching Objects to separate objects that are close together and identified as one object.
9. Click Gate Tab.



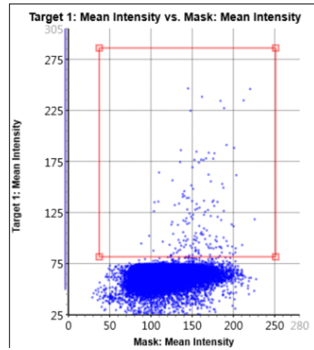
5.4 Gate Tab: Gating for Caspase 3/7 Positive Cells:

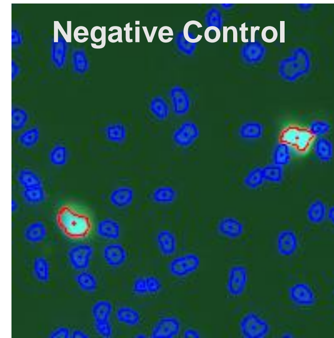
The Gate Tab allows user to specify sub-population settings that are used after feature analysis.

1. Select a well from negative control for gating.
2. Click the "+" plots button.
    a. Pick a source population. ALL is the default population that the system assigns to all the data points in the segmentation result from the analyze tab.
    b. Pick Scatter plot for plot type.
    c. Select Green mean intensity on X-axis .
    d. Select Blue mean intensity on Y-axis.
    e. Click OK.

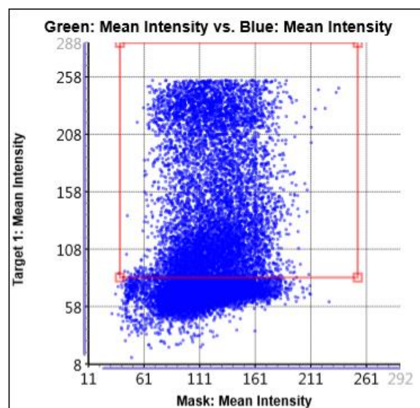


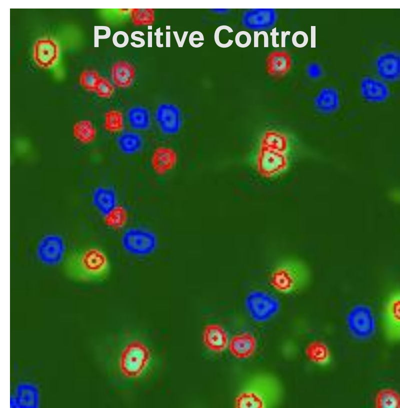
3. Create a gate on a scatter plot using the gate selection tools.
    a. Click rectangle tool, click and drag to draw a box over the dots in the scatter plot.



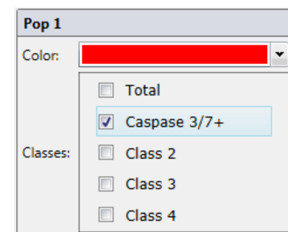


- The color of the drawn gate corresponds to the outlined cells shown in the graphic overlay. The image of outlined cells updates in real time as you select or change the selection area in the plot.
- Size of gate can be changed by holding and dragging a corner small square.
- The location of the gate can be changed by clicking center of gate and dragging.





4. Check box on left column for Caspase 3/7+ designates objects inside red gate to be reported as Caspase 3/7+.
5. Once the classification of the sub-population is defined, click Start Analysis.



5.5 Results Tab:

1. Double click on a well in the results page to view the whole-well image.
2. Scroll in to see high resolution images and graphic overlay.
3. Once data analysis is finished, click Export Well Level Data into CSV file format.
4. Data reported are Counts, Standard Deviation, Average Mean Intensities, Integrated Intensity measurements of all channels, and analysis parameters for current analysis.

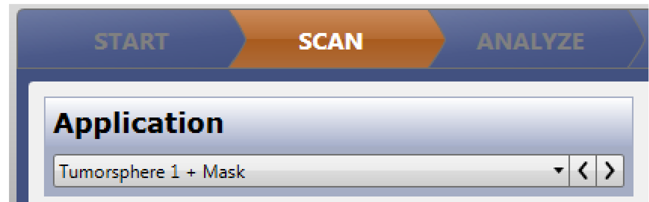
6. Celigo Instrument Parameters for 3D Caspase 3/7 Continuous Monitoring & Endpoint Detection

6.1 Start Tab:

5. Select Create a New Scan.
6. Select appropriate Plate Category and Vendor Type.
7. Type in a Plate ID, or select an old one.
8. Click Load Plate.

6.2 Scan Tab:

6.2.1 Select an application



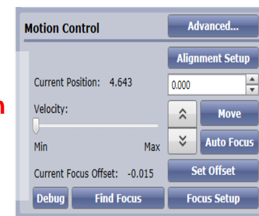
Tumorsphere 1 + 2 + 3 + Mask
Tumorsphere 1 + 2 + Mask
Tumorsphere 1 + Mask

1. Select Application Tumorsphere 1 + Mask.

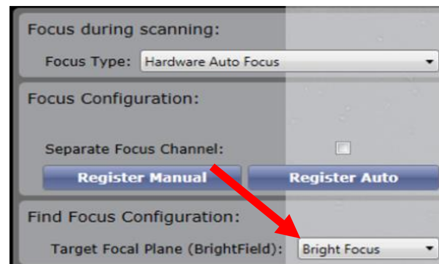
NOTE: For additional FL channels, select the addition channels, e.g. Tumorsphere 1+2+Mask, Tumorsphere 1+2+3+Mask. Follow the same set up, except change the illumination to the FL wavelength of interest. Focus in FL channels will be the same as the Bright field channel. Analysis parameters are only set up for the Bright field Mask channel.

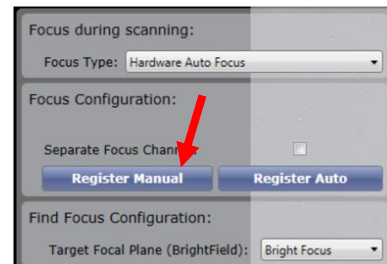
6.2.2 Setup Mask (Bright field) Channel

Plate Map Navigation Area

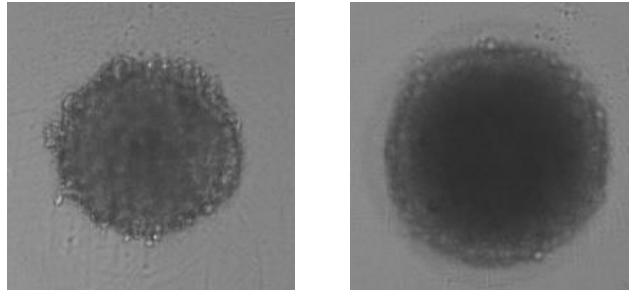


1. Select a well with sufficient amount of caspase 3/7+ (such as a treated well) by clicking on plate map navigation area.
2. Change Channel to Mask.
3. Change the illumination to Bright field.
4. Click AutoCalc to find the correct illumination to have background pixels between 120-150 relative FL units.
5. Click Focus Setup.
6. Turn on Live camera.
7. Manually adjust focus to get crisp outer edge of spheres with up/down arrows.
8. Register Manual (with Hardware Auto Focus).
9. Close dialog by clicking Focus Setup.





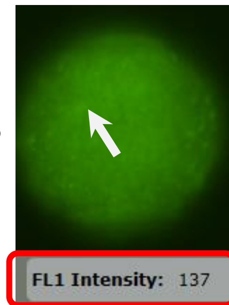
Crisp Sphere Edge (GOOD) Blurry Sphere Edge (BAD)



6.2.3 Green Channel Setup

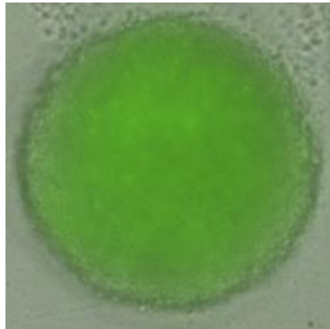
1. Change Channel to FL1.
2. Change illumination to Green fluorescence.
3. Set illumination exposure time for green channel to 30,000 µs.
   - This exposure time should correspond to objects (caspase3/7+ cells or spheres) average pixel intensity values between 100-150 relative FL units.
4. Click Selection on plate map navigation area and highlight wells for imaging.
5. Click Start Scan.

Hover Mouse over object to observe Pixel Intensity Display

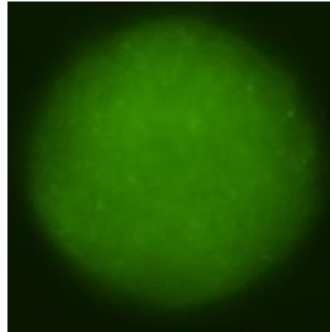


Images of different channel combinations for a drug treated Caspase 3/7 positive sphere:

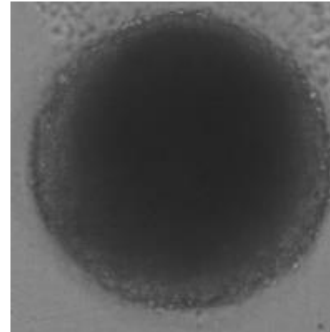
Caspase 3/7 + BF

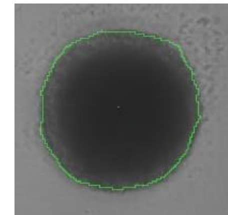


Caspase 3/7 Only



BF Only

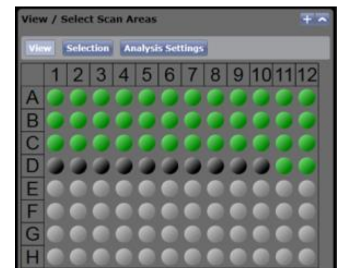




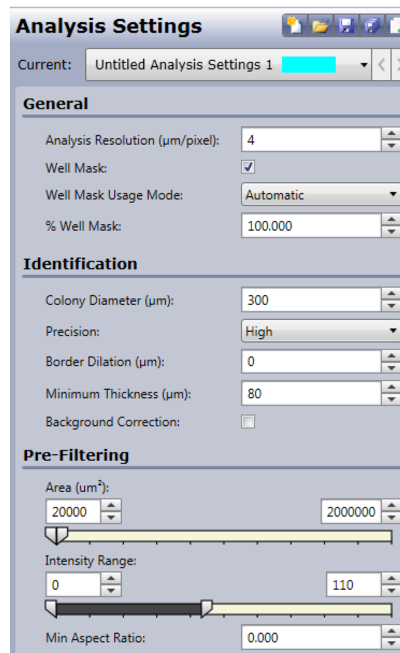
6.3 Analyze Tab:

6.3.1 "Mask" Bright field Analysis Setup

- The "Mask" channel is a single graphic overlay mask used to identify objects in one channel, typically the last channel. The Mask channel is used as a Total object count. Additional channels will use the mask channel graphic overlay to measure fluorescent and bright field intensity for each object. Therefore, analysis parameters for the mask channel are the only ones needing to be optimized in Analyze Tab.
- As images are acquired for each well, plate navigation map with show green wells for images saved to database.



1. Select a green well in plate map navigation area to view image in display area.
2. Turn On Mask Graphic Overlay, turn OFF FL1 Graphic Overlay so that you are only viewing Mask graphic overlay (green outline around sphere).
    - Light blue indicates ON
    - Dark blue indicates OFF



3. Check box ON Well Mask, to exclude object detection outside well area.
4. Select an average Colony Diameter value.
5. Adjust Precision for amount of detail needed at edge of sphere.
6. Adjust Minimum Thickness for desired roundness.
    - Increasing values will smooth out long protrusions on spheres
    - Decreasing values will create more jagged edges on sphere
7. Turn ON Background correction if needed.
8. Click Start Analysis.

6.4 Results Tab:

5. Double click on a well in the results page to view the whole-well image.
6. Scroll in to see high resolution images and graphic overlay.
7. Once data analysis is finished, click Export Well Level Data into CSV file format.
8. Data reported are well Counts, average mean intensities of all channels, integrated intensity measurements, and analysis parameters for current analysis.

7.0 Additional Resources

7.1 Citations

1. Cohen GM. (1997) Caspases: the executioners of apoptosis. *Biochem J*. 326: 1–16.
2. Cen H, et al. (2008) Devd-Nucview488: A Novel Class of Enzyme Substrates for Real-Time Detection of Caspase-3 Activity in Live Cells. *FASEB J.* 22(7):2243-2252.
3. Elmore S. (2007). Apoptosis: A Review of Programmed Cell Death. *Toxicol. Pathol*. 35(4): 495-516.
4. Rastogi RP, *et al.* (2009). Apoptosis: Molecular Mechanisms and Pathogenicity. *EXCLI Journal*. 8:155-181

7.2 Quick Reference

Image plate at the suggested following exposures to obtain object pixel intensities between 100-150: These values are not absolute and may require further optimization.

- Hoechst – Total Channel @ 80,000 µs, Focus Setup with HWAF or IBAF.
- Caspase 3/7 – Total Channel @ 30,000 µs, Focus Setup with HWAF or IBAF.

7.3 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

7.4 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.

7.5 Storage and Handling

- For long term storage, store ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture between 2°C to 8°C.
- For long term storage, store ViaStain™ Hoechst 33342 (Component B) between -16°C to -24°C. Thaw to room temperature before use.

8.0 Ordering Information

8.1 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