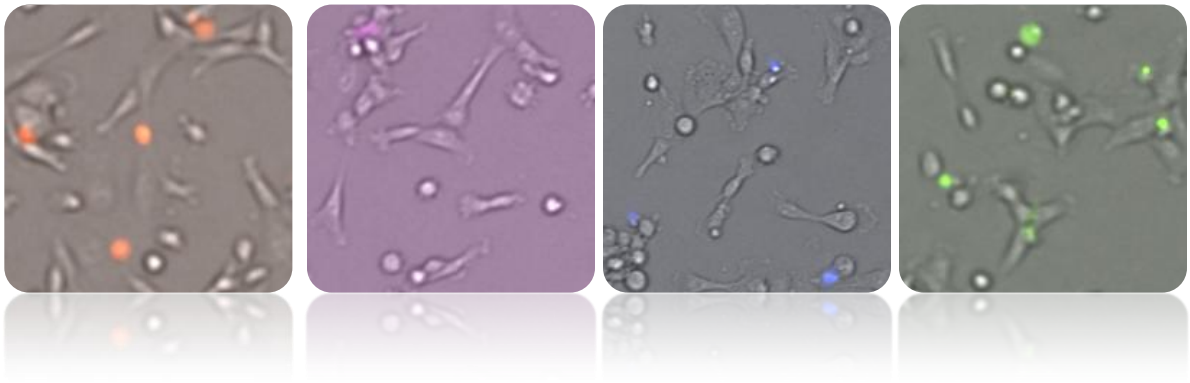
Celígo®

ViaStain™ Dead Cell Nuclear Dyes

Product Number:

CS1-V0012-1 Green
CS1-V0013-1 Red
CS1-V0014-1 Far Red
CS1-V0015-1 Blue

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



Nexcelom Bioscience

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

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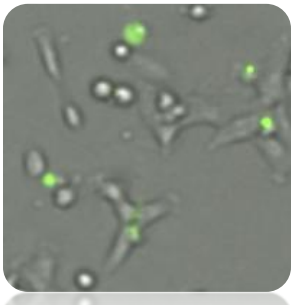
1.0 Introduction

1.1 Description of Assay



Dead Nuclear Dye:
In this viability assay, the total number of dead cells are identified by staining the cells with Dead Nuclear dye. These dyes are membrane impermeable and only enters the dead (membrane compromised) cells, where upon entry it binds to DNA in an intercalating manner. After staining the cells, the Celigo instrument can scan, analyze and report the number of dead cells on a per well basis.

1.2 Materials and Reagents

- **ViaStain™ Dead Nuclear Dye,** Store at -20˚C

Product Name	Catalog No.	Stock	Volume	No. 96-well Plates
ViaStain™ Dead Cell Nuclear Green	CS1-V0012-1	5 mM	100 µL	5
ViaStain™ Dead Cell Nuclear Red	CS1-V0013-1	5 mM	50 µL	5
ViaStain™ Dead Cell Nuclear Far Red	CS1-V0014-1	5 mM	50 µL	5
ViaStain™ Dead Cell Nuclear Blue	CS1-V0015-1	5 mM	100 µL	5

- ViaStain™ Dead Cell Nuclear Dye Instruction Booklet

1.3 Additional Equipment and Materials Required

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

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 with a total volume per well of 100 µL.

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 Benzethonium, or Staurosporine. For Celigo experiments, MDA-MB-231 cells were treated with 100 µM Benzethonium for 2 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 this example of a 96-well plate, prepare a 2X master mix using the following reagents: Diluent (cell culture media or PBS) and Dead Cell Nuclear Dye. Some media may have background auto-fluorescence with the dyes, and therefore PBS is recommended in place of media if auto-fluorescence is observed.

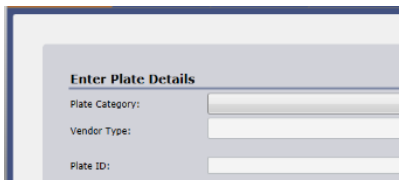
1. **Prepare a 2X Master Mix staining solution**:
    a. Pipette 10 mL of diluent (cell media or PBS) into a 15-mL centrifuge tube.
    b. Add X µL of the ViaStain™ Dead Cell Nuclear Dye to the 15-mL tube. (See table below for individual dye volumes). Cap and vortex tube for 10 seconds.
2. To wells containing 100 µL with cells, pipette 100 µL of 2X Master Mix, very gently into each well, so not to disturb lightly attached cells. Final volume per well will now be 200 µL.
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 in PBS	[2X Master Mix]	Final [ ] (in well)
ViaStain™ Dead Cell Nuclear Green	CS1-V0012-1	5 mM	20 µL	10 µM	5 µM
ViaStain™ Dead Cell Nuclear Red	CS1-V0013-1	5 mM	10 µL	5 µM	2.5 µM
ViaStain™ Dead Cell Nuclear Far Red	CS1-V0014-1	5 mM	10 µL	5 µM	2.5 µM
ViaStain™ Dead Cell Nuclear Blue	CS1-V0015-1	5 mM	20 µL	10 µM	5 µM

3.0 Celigo Image Acquisition, Analysis, and Results

3.1 Home / Setup Tab:

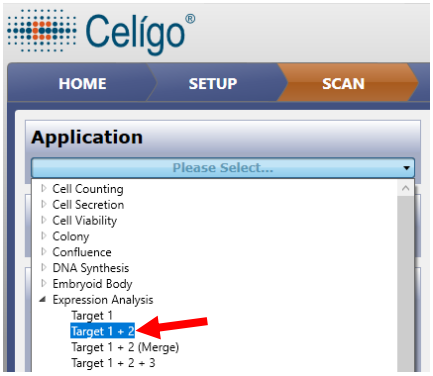
1. Select Create a New Scan
2. Select appropriate plate category and vendor type
3. Type in a Plate ID, or select an old one
4. Click Load Plate



3.2 Scan Tab:

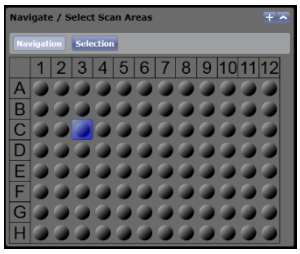
3.2.1 Target 1 Channel Setup

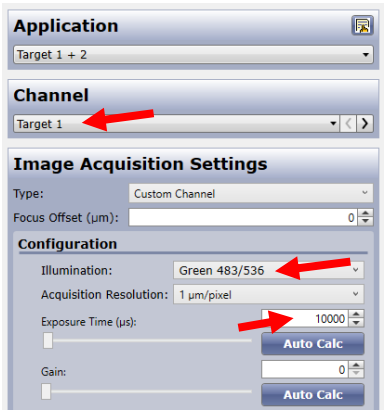
Target 1 will be the fluorescent dye and Target 2 will be for a brightfield image. Customization of the channel names can be done with the customize button. See user Guide for details on how to customize channel names.



1. Select Application Expression Analysis: Target 1+2.
2. Select a well with sufficient number of dead stained cells by clicking on plate map navigation area.

Plate Map Navigation Area



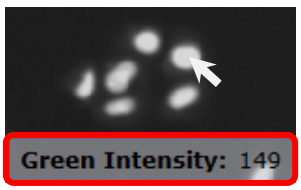


3. Change Channel to Target 1.
4. Change illumination to appropriate fluorescent color.
5. Set illumination exposure time for dead channel to appropriate exposure (µs). See suggestion in table below.

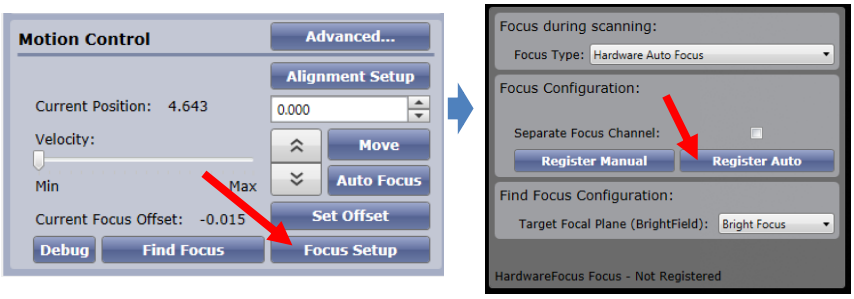
Product Name	Catalog No.	Celigo Illumination	Exposure (µs)
ViaStain™ Dead Cell Nuclear Green	CS1-V0012	Green 483/536	10,000
ViaStain™ Dead Cell Nuclear Red	CS1-V0013	Red 531/629	10,000
ViaStain™ Dead Cell Nuclear Far Red	CS1-V0014	Far Red 632/692	10,000
ViaStain™ Dead Cell Nuclear Blue	CS1-V0015	Blue 377/470	75,000

- 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



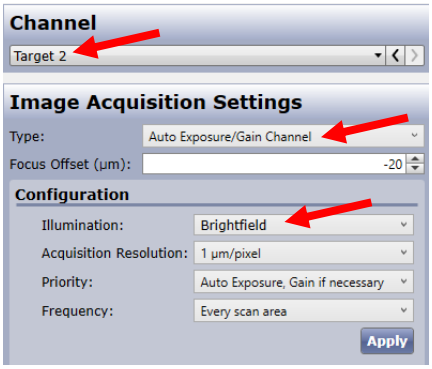
6. Click Focus Setup – Register Auto (with Hardware Auto Focus) – close dialog by clicking Focus Setup.



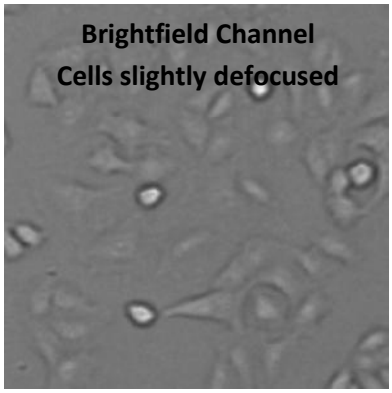
7. Close dialog by clicking Focus Setup

3.2.2 Target 2 Channel Setup

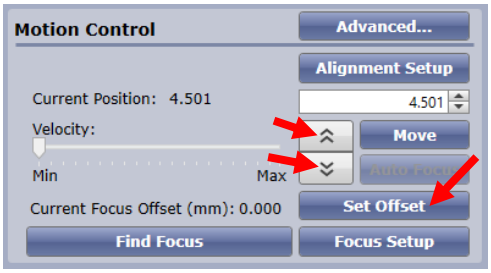
Target 1 will be the fluorescent dye and Target 2 will be for a brightfield image. Customization of the channel names can be done with the customize button. See user Guide for details on how to customize channel names



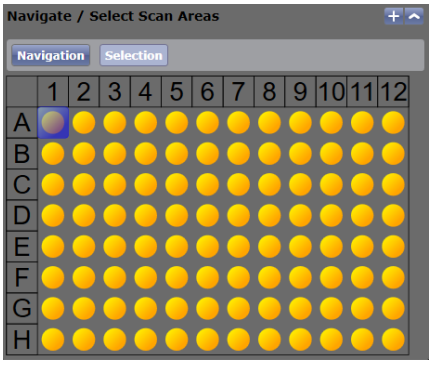
1. Change channel to Target 2.
2. Change illumination to Brightfield.
   - Set illumination exposure time Auto Exposure/Gain Channel This exposure time should correspond to objects (dead cells) average pixel intensity values between 150-175. Adjust exposure accordingly.



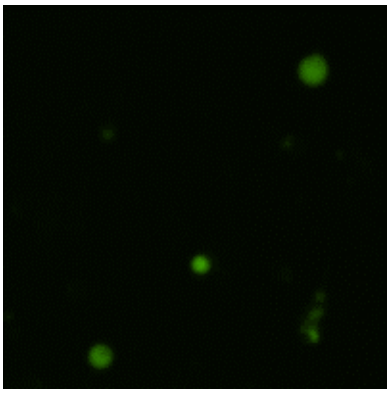
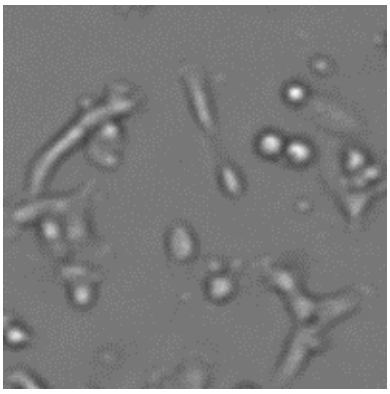
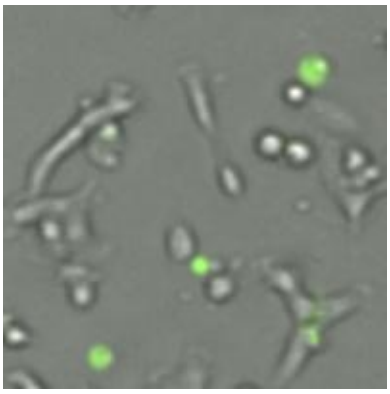
3. Click Find Focus to let the system find the correct focal plane.
4. Click Set Offset once correct focal plane was found.



5. Click Selection on plate map navigation area and highlight wells for imaging.
6. Click Start Scan.

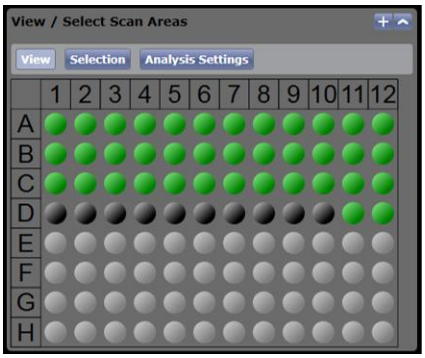


Example Images for Dead Nuclear Green and Brightfield cell

Target 1 (Green)	Target 2 (Brightfield)	Merged
		

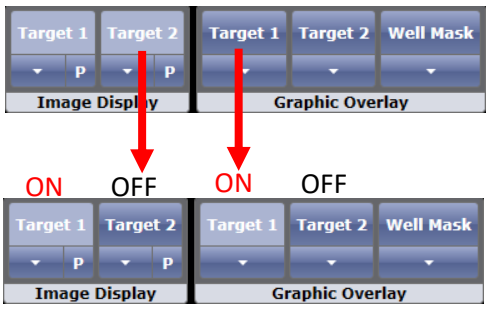
3.3 Analyze Tab:

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

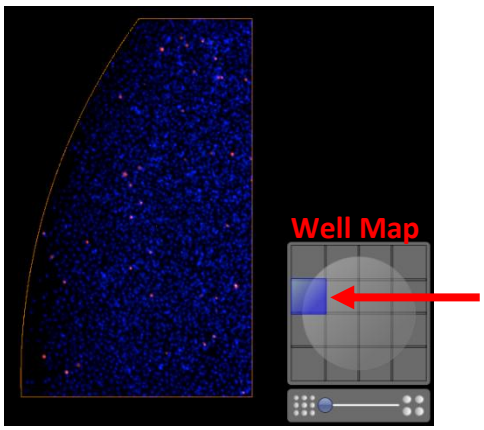


3.3.1 "Target 1" Channel Analysis Setup

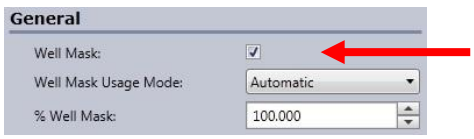
1. Turn ON Target 1 Graphic Overlay by clicking on word "Target 1"
2. Turn OFF Target 2 Image Display by clicking on word "Target 2"
   - Light blue indicates ON
   - Dark blue indicates OFF



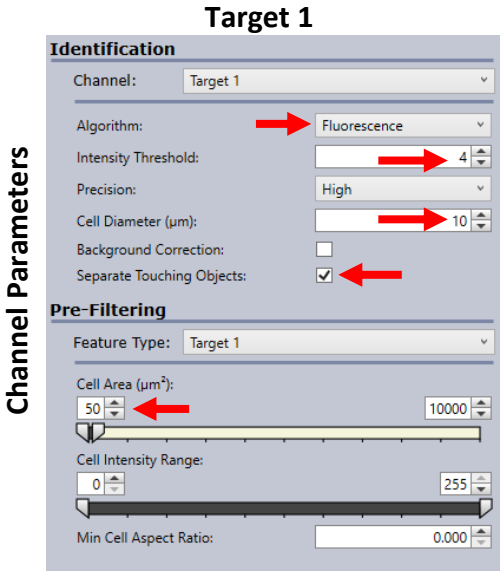
3. Click on an edge image in Well Map to view edge of well.



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

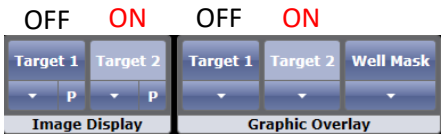


5. In Identification Channel for Target 1, select Algorithm of Fluorescence (Default)
6. Adjust Intensity Threshold value to properly detect (outline) nuclei in image (Default 4)
    - Higher values detect only brighter objects
    - Lower values will include more dim objects
7. Adjust Cell Diameter (µm) to be average expected diameter of object (Default 10)
    - Higher values will expand current objects area
    - Lower values will decrease current objects area
8. Check box Separate Touching Objects to separate nuclei that are close together and identified as one object.
9. 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

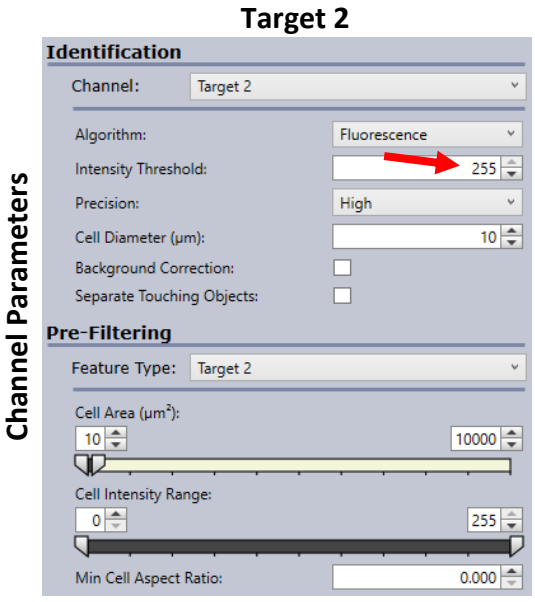


2. Turn ON Target 2 Graphic Overlay, turn OFF Target 1 Graphic Overlay so that you are only viewing Target 2 graphic overlay

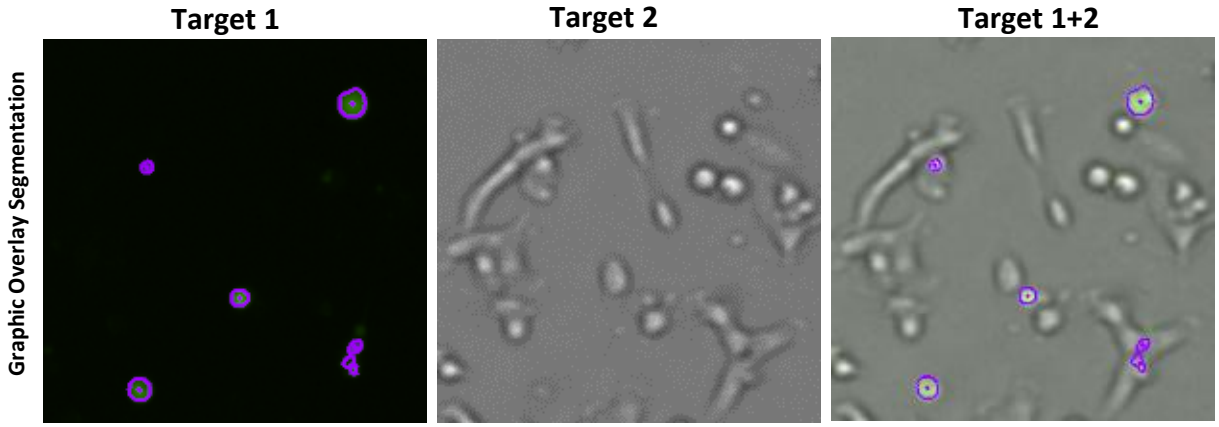
3. Change Identification channel to Target 2

4. Adjust Intensity Threshold to 255 to not detect any cells

5. Click Start Analysis



10. of interest. Suggested minimum value of 30.



Example Images for Dead 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 % 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:

- Dead 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™ Dead Cell Nuclear Green	CS1-V0012-1	Green 483/536	10,000
ViaStain™ Dead Cell Nuclear Red	CS1-V0013-1	Red 531/629	10,000
ViaStain™ Dead Cell Nuclear Far Red	CS1-V0014-1	Far Red 628/688	10,000
ViaStain™ Dead Cell Nuclear Blue	CS1-V0015-1	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