

PRODUCT INSERT

Instrument Compatibility:

Cellometer™ Spectrum, K2,
and Ascend
Cellaca™ MX and PLX

ViaStain™ Calcein-AM/PI Cell Viability Kit

Part number: CSK-0118

Test number: 100 Tests

Storage: 2 to 8 °C (Propidium Iodide)
-16 to -24 °C (Calcein AM)

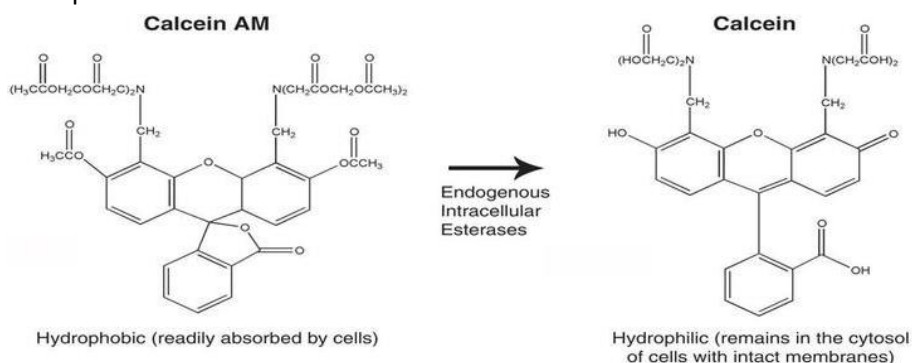
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1 Introduction

1.1 Assay Description

Calcein AM (Calcein acetoxymethyl ester) is a cell permeable, non-fluorescent compound. Upon crossing the cell membrane, calcein AM is rapidly hydrolyzed by cellular esterases inside live cells. The hydrolysis cleaves the AM group, converting the non-fluorescent calcein AM to a bright green fluorescing calcein. The newly created calcein is more hydrophilic and becomes trapped inside the cells with intact membranes (1). Cells that do not possess active cytoplasmic esterases are unable to convert calcein AM to calcein and therefore do not fluoresce green. This allows for a quick and easy detection of metabolically active cells in a sample.



Stains such as propidium iodide (PI), 7-AAD, and ethidium bromide (EB), are membrane exclusion dyes that are frequently used to stain non-viable nucleated cells with compromised membranes. Acridine orange freely diffuses across the cell membrane and stains DNA in all nucleated cells. When AO and PI are combined it is possible to determine percentage viability for nucleated cells. When Calcein AM is used in conjunction with PI, it is possible to determine the percentage of vitality/viability based on the number of metabolically active (green fluorescent) and non-viable (red fluorescent) cells in a sample.

Since calcein AM does not require DNA binding, it stains all metabolically active cells and can be used to measure metabolic activity in non-nucleated cells, such as platelets (2). Calcein-AM is also a good alternative for analysis of adipocytes, as the AO dye has shown some non-specific binding of lipid droplets that does not occur with calcein AM (3). Because calcein AM is photostable, shows low cytotoxicity, does not affect cellular functions, and requires cellular esterases for conversion to green-fluorescing calcein, it is a popular stain for the examination of cell vitality and viability. (1,4,5).

1. Braut-Boucher, F. *et al. Journal of Immunological Methods*. Vol. 178, Issue 41 (1995).
2. Verheul, HW. *et al. Blood*. Vol. 96, No. 13 (2000).
3. Kilroy, G. *et al. PLoS One*. Vol. 4, Issue 9 (2009).
4. Luc S. De Clerck. *et al. Journal of Immunological Methods*. Vol. 172, Issue 1 (1994).
5. Parish, CR. *Immunology and Cell Biology*. Vol. 77 (1999).

1.2 Materials Included

Reagents	Volume	Catalog number	Number of Tests
Propidium Iodide Staining Solution (Component A)	500 µL	CSK-0118	100 Tests
Calcein AM Staining Solution (Component B)	200 µL		

1.3 Materials Required

- Microcentrifuge tube
- Pipette and pipette tips
- Distilled H₂O (dH₂O)
- Phosphate Buffered Saline (1X PBS) **OR** cell culture media
- Trypsin EDTA (if working with adherent cells)
- Disposable counting slide or plate, depending on cell counter/image cytometer:
 - Cellometer counting slide (Cat. # SD100 or PD100) for Cellometer K2 and Spectrum
 - Ascend counting slide (Cat. # ASD-CHM3 or ASD-CHM8) for Cellometer Ascend
 - Counting plate (Cat. # CHM24-A100 or CHM24-B100) for Cellaca systems

1.4 Compatible Instruments and Software Requirements

- Cellometer K2 with Cellometer K2 or Cellometer Matrix™ software
- Cellometer Spectrum with Fluorescence Optics Modules S1-534-470 and S1-655-527 with Cellometer Spectrum Software
- Cellaca MX High-throughput Cell Counter with Matrix™ software
- Cellaca PLX High-throughput Image Cytometer with Matrix™ software

2 Assay Protocol

2.1 Cell Preparation for Staining

2.1.1 Suspension Cells

1. Prepare cell sample at $2-3 \times 10^6$ cells/mL.
 - a. Dilute or concentrate as needed.

2.1.2 Adherent Cells

1. Using 1X Trypsin EDTA, trypsinize cells until they have lifted off the plate (~15 min).
2. Prepare cell sample at $2-3 \times 10^6$ cells/mL.
 - a. If available, use the Cellometer Sample Adjustment Calculator to determine the sample volume required to obtain desired concentration.
3. Centrifuge cells at $300 \times g$ for 5 min.
4. Remove the supernatant and re-suspend cells in 1 mL of 1X PBS or cell culture media.

2.1.3 Biological Samples

1. If using whole blood, cord blood, or bone marrow no additional preparation is required.

2.2 Preparation of Calcein AM Working Solution

1. Pipette 2 μ L Calcein AM (**Component A**) into 18 μ L of dH₂O to prepare a Calcein AM Working Solution.
2. Mix by pipetting up and down at least 15 times or vortexing.

NOTE: Solution is stable up to 3 h.

2.3 (optional) Generation of a Calcein AM Negative, PI Positive Control

1. Heat-kill cells by placing in a boiling water bath for 10 min.
2. Allow them to cool to room temperature.
3. Mix live and heat killed dead cells at a desired ratio or use the control as is.

NOTE: If using the control as is on the Cellometer Ascend or Cellaca MX/PLX, verify that it is set to focus in brightfield or FL2 (PI channel), since the green calcein signal (FL1 channel) will be absent, and the instrument will not have anything to focus on.

2.4 Staining Procedure

When staining cultured cells (suspension or adherent), prepare samples according to Section 2.4.1 below. For biological samples, such as whole blood, cord blood, or bone marrow, prepare according to Section 2.4.2.

2.4.1 Staining Procedure for Cultured Cells

1. Add 40 μL of cell sample to a microcentrifuge tube.
2. Add 5 μL of Calcein-AM Working Solution and 5 μL of PI (**Component B**) to the tube containing cells.
3. Gently pipette the sample up and down 10 times.
4. Incubate for 20 min at 37 °C in the dark.
5. Proceed to imaging and data acquisition according to cell counter being used:
 - a. Cellometer K2: Section 3
 - b. Cellometer Ascend: Section 4
 - c. Cellaca MX or PLX: Section 5
 - d. Cellometer Spectrum: Section 6

2.4.2 Staining Procedure for Biological Samples

1. Pipette 10 μL of fresh blood sample into 70 μL of 1X PBS.
2. Pipette 40 μL of the diluted blood sample into a new microcentrifuge tube.
3. Add 5 μL of Calcein AM Working Solution and 5 μL of PI (**Component B**) to the cell sample.
4. Gently pipette the sample up and down 10 times.
5. Incubate for 20 min at 37 °C in the dark.
6. Proceed to imaging and data acquisition according to cell counter being used:
 - a. Cellometer K2: Section 3
 - b. Cellometer Ascend: Section 4
 - c. Cellaca MX or PLX: Section 5
 - d. Cellometer Spectrum: Section 6

3 Cellometer K2 Imaging and Data Acquisition

1. Gently mix the sample by pipetting up and down at least 10 times.
2. Pipette 20 μ L of each sample into a counting chamber.
NOTE: *If using SD100 slides, peel plastic film off both sides before loading.*
3. Select the appropriate assay for Calcein AM/PI vitality measurement.
NOTE: *If assay is unavailable, please contact support at USCAN.service@revvity.com.*
4. Verify that the dilution factor is set to 1.25.
5. Insert slide into the Cellometer K2 instrument.
6. Preview brightfield and fluorescent images. Focus, if necessary.
7. Click **Count**.

4 Cellometer Ascend Imaging and Data Acquisition

1. Gently mix the sample by pipetting up and down at least 10 times.
2. Pipette 20 μ L of each sample into a counting chamber.
NOTE 1: *Peel plastic film off both sides before loading.*
NOTE 2: *Loading volume for Cellometer Ascend 8-chamber slides is 10 μ L.*
3. Select the appropriate assay for Calcein AM/PI vitality measurement in the Matrix software.
4. Verify that the dilution factor is set to 1.25.
5. Insert slide into the Cellometer Ascend to **Preview** or **Count**.
 - a. If **Skip Preview** is set to **No**, software will go into preview mode where fluorescent exposures can be adjusted.
 - b. If **Skip Preview** is set to **Yes**, software will immediately proceed to counting sample.

5 Cellaca Imaging and Data Acquisition

1. Gently mix the sample by pipetting up and down at least 10 times.
2. Pipette 50 μ L of each sample into a loading well of the counting plate.
3. Load plate into the Cellaca instrument with A1 in the top left corner.
4. Select the appropriate assay for Calcein AM/PI vitality measurement in the Matrix software.
5. Verify that the dilution factor is set to 1.25.
6. Click **Preview**.
7. Click **Auto Focus**, if cells are not in focus.
8. Preview brightfield and fluorescent images and adjust fluorescent exposures, as needed.
9. Click **Count**.

6 Cellometer Spectrum Imaging and Data Acquisition

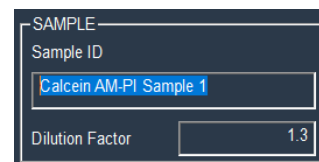
Prior to data acquisition it is always advised to review the auto save set-up to make sure the acquired data is properly saved (see Section 7.2).

1. Gently mix the cell sample by pipetting up and down at least 10 times.
2. Load 20 µL of stained sample into a Cellometer counting chamber.
3. Insert the slide into the Cellometer instrument.
4. Wait 60 sec for the cells to settle in the chamber.
5. Type a name for your sample into the Sample ID text box.
6. Verify the default dilution factor:

- a. For cultured cells, the dilution is 1.25.

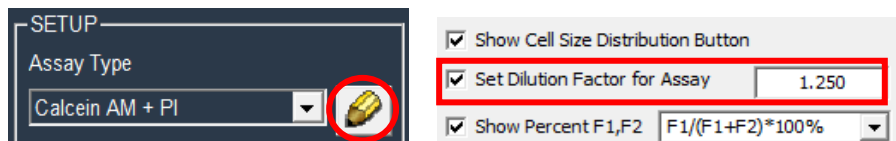
NOTE: The dilution gets rounded up on the main software screen and is displayed as 1.3.

- b. For Biological Samples (Calcein AM + PI **CS**) the dilution is 10.



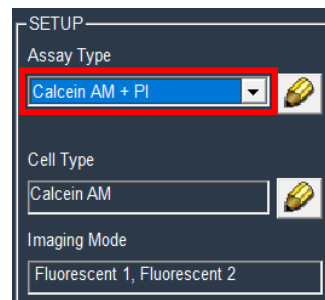
NOTE 1: If additional dilutions were performed during sample preparation, adjust the dilution factor accordingly.

NOTE 2: To adjust the dilution factor, click on the pencil icon, locate the “Set Dilution Factor for Assay” in the dialog pop-up screen, and type in the value.

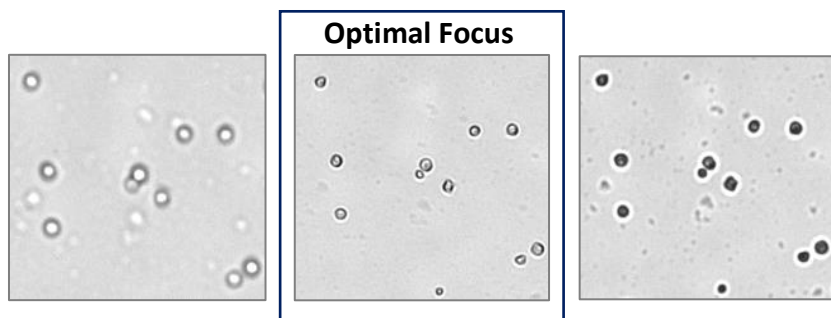


7. Select **Calcein AM + PI** or **Calcein AM + PI_CS** from the Assay drop-down menu.

- a. If assay is not present in the drop-down menu, import it (see Section 8.2).
- b. Assay should be run using default settings (see Section 7.3).
- c. Section 7.4 shows user how to create Calcein AM + PI for Biological Samples (Calcein AM + PI_CS) using the Calcein AM + PI assay.



8. Click Preview B1 at the bottom left of the main software screen.
9. Adjust focus for the bright field image using the focus knob.
 - a. Cells in focus will have a bright center and dark outline with a crisp contrast between background and the cell membrane. See Focus Guide below.

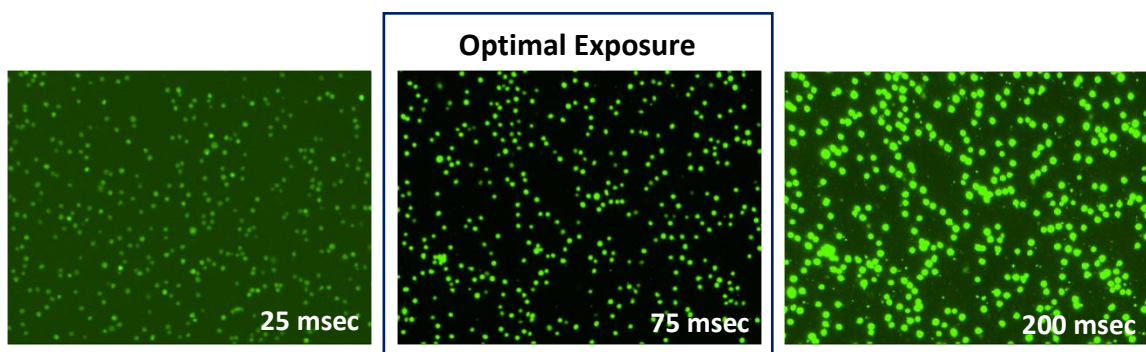
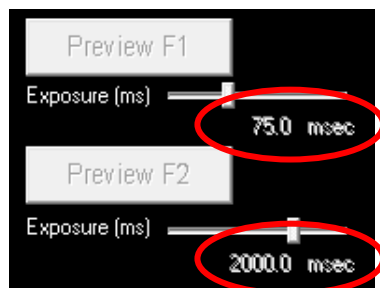


10. Click Stop Preview.

11. Click Preview F1 button and verify that the fluorescence signal displays as 100% of range.

- a. Default exposure time for F1 (Calcein AM) is 75 msec for cultured samples and 200 msec for biological samples.

- i. Optimal exposure time will generate a bright image with well-defined fluorescent spots (75 msec; see focus guide below).
- ii. Under-exposure will yield dark images with weak spots demonstrating insufficient fluorescent signal (25 msec).
- iii. Over-exposure will yield images that are too bright with fluorescent spots that are large and sometimes overlapping (200 msec). Spots are also less distinct from background.



12. Click Preview F2.

- a. Default exposure time for F2 (PI) is 2,000 msec for cultured samples and 4,000 msec for biological samples.

13. Click the Count button at the bottom of the screen.

14. When counting is complete, the Results Table will appear on the screen.

- a. The total cell concentration should be between 3×10^5 and 1×10^7 cells/mL (see example in the report below). Recount a concentrated or diluted cell sample as needed.

The default report displays:

Counting Results			×
Assay: Calcein AM + PI		Date: 09/30/2024 09:48:03	Show Size Distribution
Cell Type F1: Calcein AM			
Cell Type F2: PI for Calcein AM			
Sample ID: Dry Dye_4C_1_20 200_20230608_142812-2			
Dilution Factor: 1.30			
Results:			
Count	Concentration	Mean Diameter	
=====	=====	=====	
Total cells: 1612	2.96x10 ⁶ cells/mL	9.8 microns	
Calcein positive: 971	1.78x10 ⁶ cells/mL	9.0 microns	
PI positive: 641	1.18x10 ⁶ cells/mL	10.9 microns	
Vitality/Viability: 60.1%			

- **Calcein positive** (metabolically-active) cell counts, concentration, and mean cell diameter.

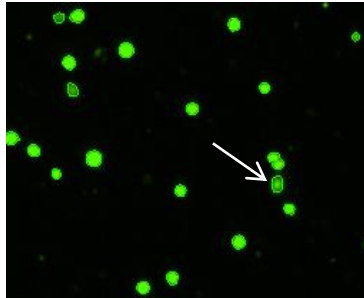
- **PI-positive** (non-viable) cell counts, concentration, and mean cell diameter.
- **Total cell** counts, concentration, and mean cell diameter.
- **Vitality/Viability %** of the cell sample:
$$\frac{\text{Calcein positive cells}}{\text{Calcein positive cells} + \text{PI positive cells}}$$

15. Click the **Close** button at the bottom right corner of the Counting Results table.

16. Select the F1 Image and check Counted at the right-hand side of the screen.

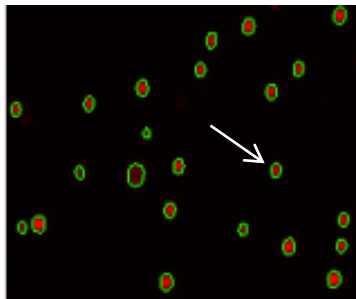
17. Review the counted image to confirm that the Calcein-positive cells are counted correctly.

- Individual cells within clumps should be circled in green, indicating that they are being counted individually.



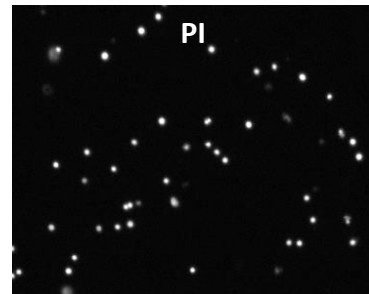
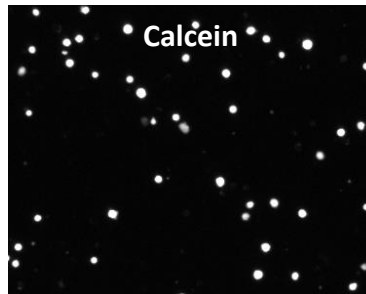
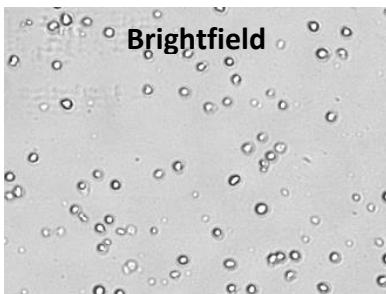
18. Click F2 Image to confirm that the PI-stained cells are being counted correctly as well.

NOTE: If cells are not being counted correctly, please contact Revvity Technical Support for assistance with optimization of counting parameters (see Section 11).



19. To confirm that data has been saved appropriately, open the file location to check for brightfield and fluorescent cell images.

- Saved **Raw** images may be opened in the Cellometer software for re-analysis.

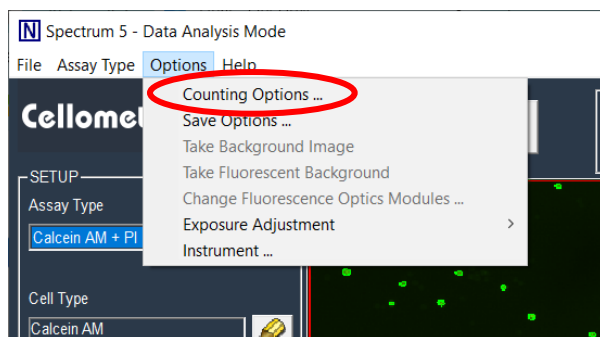


NOTE: A screen-capture software may be used to save both uncounted and counted colored fluorescent images for presentation and publication.

7 Cellometer Spectrum Software Settings

7.1 Counting Options Screen

1. Click on the **Options** Tab and select **Counting Options**....
 - a. Default settings for the Calcein AM/PI assay are shown.



Counting Options

☒ Count All

☐ Speed Count

☐ Acquire F1 first, then F2

☒ Continuous Fluorescence Preview

☐ Show Acquire Button

☐ Intelligent File Load

☐ Flat Field Fluorescent images

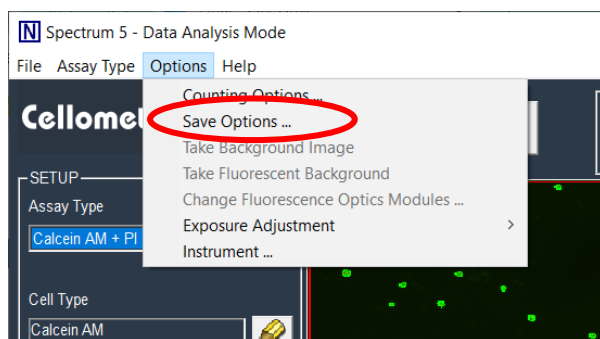
Start Program with a Dilution of

☐ Display concentration in million cells/ml format

Save Cancel

7.2 Save Options Screen

2. Click on the **Options** Tab and select **Save Options**....
 - a. Default settings for the Calcein AM/PI assay are shown.



Save Options

☐ Set sample ID as cell type

☒ Time stamp sample ID

☐ Auto increment sample ID

☐ Include instrument ID in file

☐ Log user name

Raw image file format

Counted image file format

☒ Auto save data.txt

☐ Create new Data.txt with each Sample

File: C:\Users\FrancC26325\OneDrive - R Set File

☐ Auto export Nexcelom Data Package

Folder: C:\Users\FrancC26325\OneDrive Set Folder

☒ Auto save sample image

☒ Save raw images

☐ Save JPG copies (cannot be reloaded)

☐ Save counted images

☐ Save combined images

Folder: C:\Users\FrancC26325\OneDrive Set Folder

☐ Auto export sample to Excel

Folder: C:\Users\FrancC26325\OneDrive Set Folder

☐ Auto export sample to PDF

Folder: C:\Users\FrancC26325\OneDrive Set Folder

☐ Auto print count results

Save Cancel

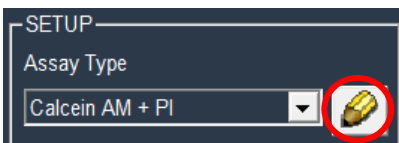
- b. Images are only saved if “Auto save data.txt” is selected in the save options menu.
- c. Saved **Raw** images may be opened in the Cellometer software for re-analysis.

7.3 Default Calcein AM/PI Software Settings

7.3.1 Dialog Screen Settings

These default software settings should be used to review or create the Calcein AM + PI assay. Detailed instructions on how to create a novel assay are located in the user manual.

1. Click on the pencil icon under Assay on the main software screen.



2. Default Dialog Settings are shown.

Assay Type

Assay Name: Calcein AM + PI ☐ Special Cells

☐ Save as New Assay Type ☐ Lock Assay from future editing

Description: Calcein AM Vitality + Propidium Iodide

Imaging Mode: Fluorescence 1 (F1) & Fluorescence 2 (F2) ☒ Acquire Brightfield Image

☐ Two Chamber Assay ☐ Multimode FL Counting

F1 Image

Cell Type: Calcein AM

Description:

Fluorophore: Calcein AM S1-534-470

Fluorescent Exp: 75.0 msec Optics Module

☐ Use Br Exp Factor of 1.0

☐ Remove FL Pos from BR count 10.0

F2 Image

Cell Type: PI for Calcein AM

Description:

Fluorophore: PI S1-655-527

Fluorescent Exp: 2000.0 msec Optics Module

☐ Use Br Exp Factor of 1.0

☐ Remove FL Pos from BR count 10.0

☐ Show Data File Buttons

☐ Show Sample Adjustment Button

☒ Show Cell Size Distribution Button

☒ Set Dilution Factor for Assay 1.250

☒ Show Percent F1,F2 $F1/(F1+F2)*100\%$ ☒ Use Custom Label Vitality

Data.txt Template: <Default Template>

Result Template: S5_Calcein AM_PI.rlt_tm

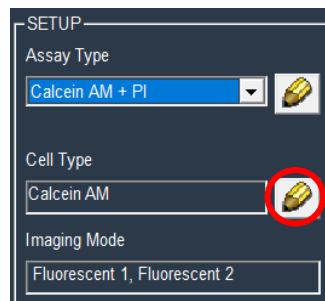
Print Template: S5_Calcein AM_PI.prn_tm

PDF Template: S5_Calcein AM_PI.pdf_tm

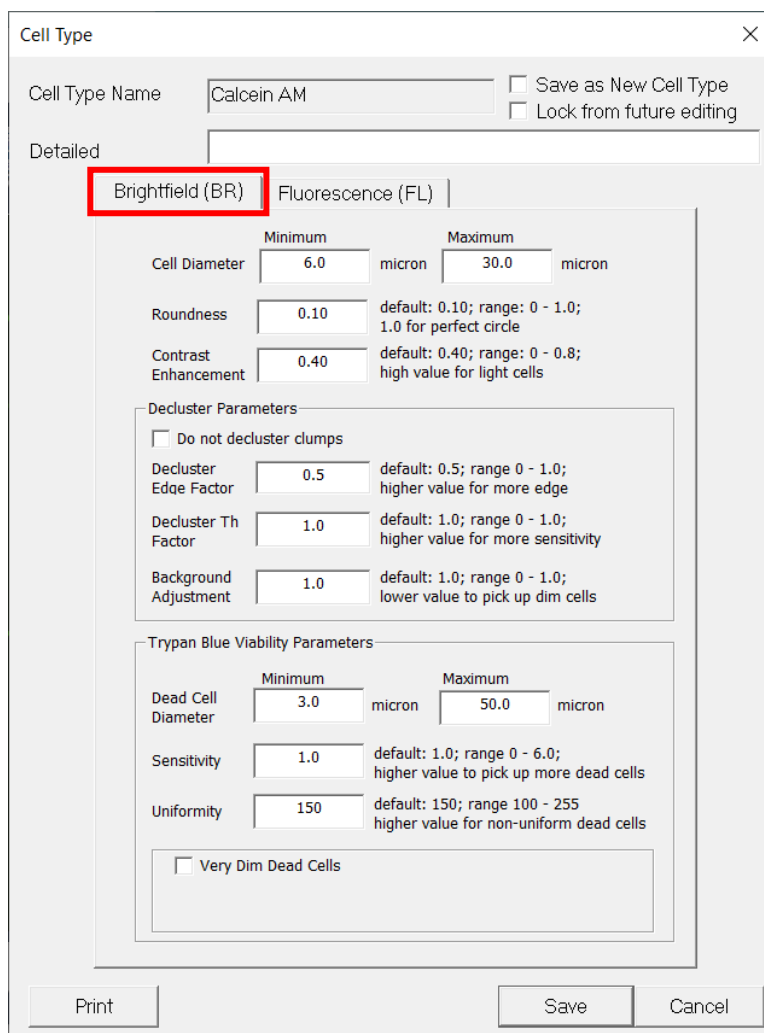
FCS Layout File: <None Selected>

7.3.2 Cell Type Settings

1. Click on the pencil icon under Cell Type on the main software screen.



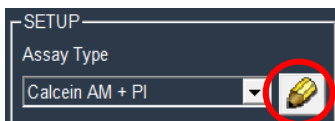
2. Default Brightfield (BR) tab Settings for the F1 image (Calcein) are shown.

A screenshot of the 'Cell Type' dialog box. At the top, 'Cell Type Name' is 'Calcein AM'. There are checkboxes for 'Save as New Cell Type' and 'Lock from future editing'. Below is a 'Detailed' section with two tabs: 'Brightfield (BR)' (highlighted with a red box) and 'Fluorescence (FL)'. Under 'Brightfield (BR)', there are settings for 'Cell Diameter' (Minimum: 6.0, Maximum: 30.0, both in microns), 'Roundness' (0.10, default: 0.10, range: 0 - 1.0), and 'Contrast Enhancement' (0.40, default: 0.40, range: 0 - 0.8). Below these are 'Decluster Parameters' with a checkbox 'Do not decluster clumps' and settings for 'Decluster Edge Factor' (0.5), 'Decluster Th Factor' (1.0), and 'Background Adjustment' (1.0). At the bottom are 'Trypan Blue Viability Parameters' with settings for 'Dead Cell Diameter' (Minimum: 3.0, Maximum: 50.0, both in microns), 'Sensitivity' (1.0), and 'Uniformity' (150). There is also a checkbox for 'Very Dim Dead Cells'. At the bottom of the dialog are 'Print', 'Save', and 'Cancel' buttons.

- Click on the Fluorescence (FL) tab.
- Default Fluorescence (FL) tab Settings for the F1 image (Calcein AM) are shown.

The 'Cell Type' dialog box is shown with the 'Fluorescence (FL)' tab selected. The 'Cell Type Name' is 'Calcein AM'. Under 'Detailed', the 'Brightfield (BR)' tab is also visible. The 'Description' is 'Calcein AM'. The 'Cell Diameter' is set to a minimum of 6.0 microns and a maximum of 30.0 microns. The 'Roundness' is 0.10. The 'Fluorescence Threshold Parameters' section shows 'Auto Threshold Fluorescent' selected with a value of 10.0, and 'Manual Threshold Fluorescent' selected with a value of 20.0. The 'Decluster Th Factor' is 1.00. Buttons for 'Print', 'Save', and 'Cancel' are at the bottom.

- Click on the pencil icon under Assay on the main screen.
- Click the Edit button on the right-hand side of the dialog box to edit F2 Image Cell Type.



The 'Assay Type' dialog box is shown with the 'F2 Image Cell Type' tab selected. The 'Assay Name' is 'Calcein AM + PI'. The 'Description' is 'Calcein AM Vitality + Propidium Iodide'. The 'Imaging Mode' is 'Fluorescence 1 (F1) & Fluorescence 2 (F2)'. The 'F1 Image Cell Type' is 'Calcein AM'. The 'F2 Image Cell Type' is 'PI for Calcein AM'. The 'F2 Image' section shows 'Description: PI', 'Fluorophore: S1-655-527', and 'Fluorescent Exp: 2000.0 msec'. The 'Edit ...' button for the F2 Image Cell Type is highlighted with a red box. Buttons for 'Save' and 'Cancel' are at the bottom.

7. Default Brightfield (BR) tab Settings for the F2 image (PI) are shown.

Cell Type

Cell Type Name

PI for Calcein AM

☐ Save as New Cell Type
☐ Lock from future editing

Detailed

Brightfield (BR)

Fluorescence (FL)

Minimum

Maximum

Cell Diameter

6.0

micron

30.0

micron

Roundness

0.10

default: 0.10; range: 0 - 1.0;
1.0 for perfect circle

Contrast Enhancement

0.40

default: 0.40; range: 0 - 0.8;
high value for light cells

Decluster Parameters

☐ Do not decluster clumps

Decluster Edge Factor

0.5

default: 0.5; range 0 - 1.0;
higher value for more edge

Decluster Th Factor

1.0

default: 1.0; range 0 - 1.0;
higher value for more sensitivity

Background Adjustment

1.0

default: 1.0; range 0 - 1.0;
lower value to pick up dim cells

Trypan Blue Viability Parameters

Minimum

Maximum

Dead Cell Diameter

3.0

micron

50.0

micron

Sensitivity

1.0

default: 1.0; range 0 - 6.0;
higher value to pick up more dead cells

Uniformity

150

default: 150; range 100 - 255
higher value for non-uniform dead cells

☐ Very Dim Dead Cells

Print

Save

Cancel

8. Click on the Fluorescence (FL) tab.
9. Default Fluorescence (FL) tab Settings for the F2 image (PI) are shown.

The screenshot shows the 'Cell Type' dialog box with the 'Fluorescence (FL)' tab selected. The 'Cell Type Name' is 'PI for Calcein AM'. The 'Detailed' section shows 'Brightfield (BR)' and 'Fluorescence (FL)' tabs, with 'Fluorescence (FL)' highlighted. The 'Description' is 'Propidium Iodide (PI)'. The 'Cell Diameter' settings are: Minimum 4.0 micron, Maximum 30.0 micron. There are checkboxes for 'Normalize intensity for cell size' and 'Non-uniform cell fluorescence'. The 'Roundness' is set to 0.10, with a default of 0.10 and a range of 0 - 1.0; 1.0 for perfect circle. There are checkboxes for 'Do not count free nuclei' and 'Advanced BR/F mode'. The 'Fluorescence Threshold Parameters' section has two options: 'Auto Threshold Fluorescent' (radio button) and 'Manual Threshold Fluorescent' (radio button, selected). Both have a value of 10.0. The 'Auto' option has a note: '* Count range 0 - 100% of brightest cell'. The 'Manual' option has a note: '* Count range 0 - 100% of image max. Lower values count dimmer cells'. The 'Decuster Th Factor' is set to 1.00, with a default of 0.9 and a range of 0 - 1.0; lower value for better decluster. At the bottom are 'Print', 'Save', and 'Cancel' buttons.

Cell Type

Cell Type Name: PI for Calcein AM ☐ Save as New Cell Type
☐ Lock from future editing

Detailed

Brightfield (BR) Fluorescence (FL)

Description: Propidium Iodide (PI)

Cell Diameter: Minimum 4.0 micron Maximum 30.0 micron
☐ Normalize intensity for cell size
☐ Non-uniform cell fluorescence

Roundness: 0.10 default: 0.10 range: 0 - 1.0; 1.0 for perfect circle
☐ Do not count free nuclei ☐ Advanced BR/F mode

Fluorescence Threshold Parameters

☐ Auto Threshold Fluorescent
10.0 * Count range 0 - 100% of brightest cell

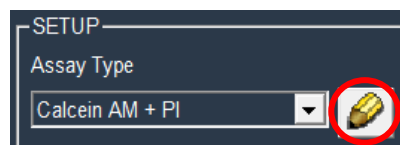
☒ Manual Threshold Fluorescent
10.0 * Count range 0 - 100% of image max
Lower values count dimmer cells

Decuster Th Factor: 1.00 default: 0.9; range 0 - 1.0; lower value for better decluster

Print Save Cancel

7.4 Software Settings for Biological Samples

1. Click on the pencil icon under Assay on the main screen.
2. Adjust the current settings.
 - a. Check the **Save as New Assay Type** box.
 - b. Rename Assay to **Calcein AM + PI_CS**.
 - c. Change the **Fluorescent Exp** for Calcein AM to 200.
 - d. Change the **Fluorescent Exp** for PI to 4000.
 - e. Change the **Dilution Factor for Assay** to 10.
 - f. Click Save.



Assay Type

Assay Name: Calcein AM + PI_CS ☐ Special Cells

☒ Save as New Assay Type ☐ Lock Assay from future editing

Description: Calcein AM Vitality + Propidium Iodide

Imaging Mode: Fluorescence 1 (F1) & Fluorescence 2 (F2) ☒ Acquire Brightfield Image

☐ Two Chamber Assay ☐ Multimode FL Counting

F1 Image

Cell Type: Calcein AM

Description: Calcein AM S1-534-470

Fluorescent Exp: 200.0 msec Optics Module

☐ Use Br Exp Factor of 1.0

☐ Remove FL Pos from BR count 10.0

F2 Image

Cell Type: PI for Calcein AM

Description: PI S1-655-527

Fluorescent Exp: 4000.0 msec Optics Module

☐ Use Br Exp Factor of 1.0

☐ Remove FL Pos from BR count 10.0

☐ Show Data File Buttons

☐ Show Sample Adjustment Button

☒ Show Cell Size Distribution Button

☒ Set Dilution Factor for Assay: 10.000

☒ Show Percent F1,F2: F1/(F1+F2)*100% ☒ Use Custom Label: Vitality

Data.txt Template: <Default Template>

Result Template: S5_Assay_Results.rlt_tm

Print Template: S5_Assay_Results.prn_tm

PDF Template: <Default Template>

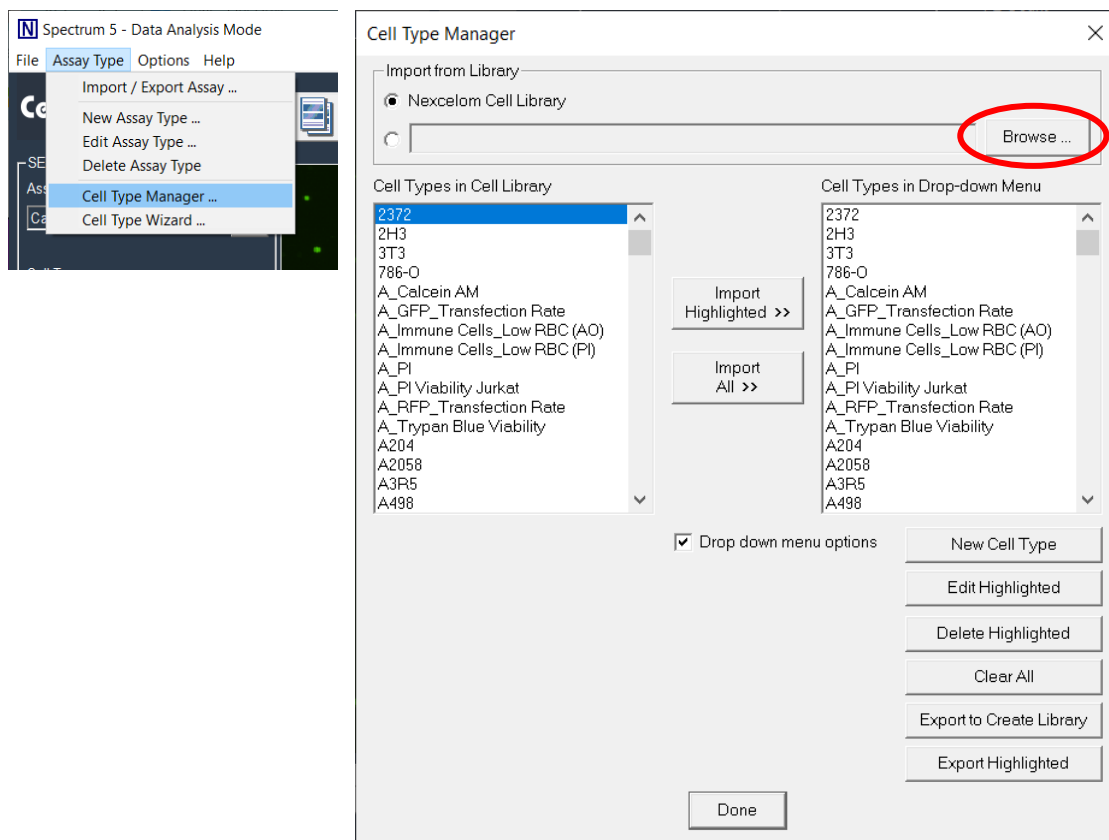
FCS Layout File: <None Selected>

3. The Calcein AM + PI_CS assay will now be available from the dropdown menu. Proceed with data acquisition of biological samples as outlined in Section 6.

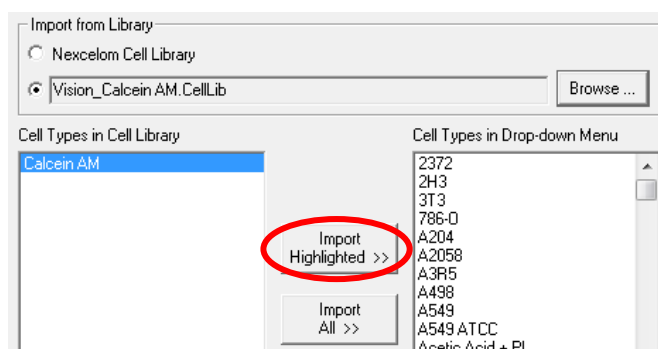
8 Cellometer Spectrum: Importing a Cell Type, Assay, or Template

8.1 Importing a Cell Type

1. Locate and click on the “Assay Type” at the top of the screen. Followed by Cell Type Manager.
2. Once the Cell Type Manager appears, click on Browse and navigate to the location of the cell type file (will end with “.CellLib”). For the Calcein AM/PI assay, there is a Vision_Calcein AM.CellLib that can be requested through Revvity Customer Support.



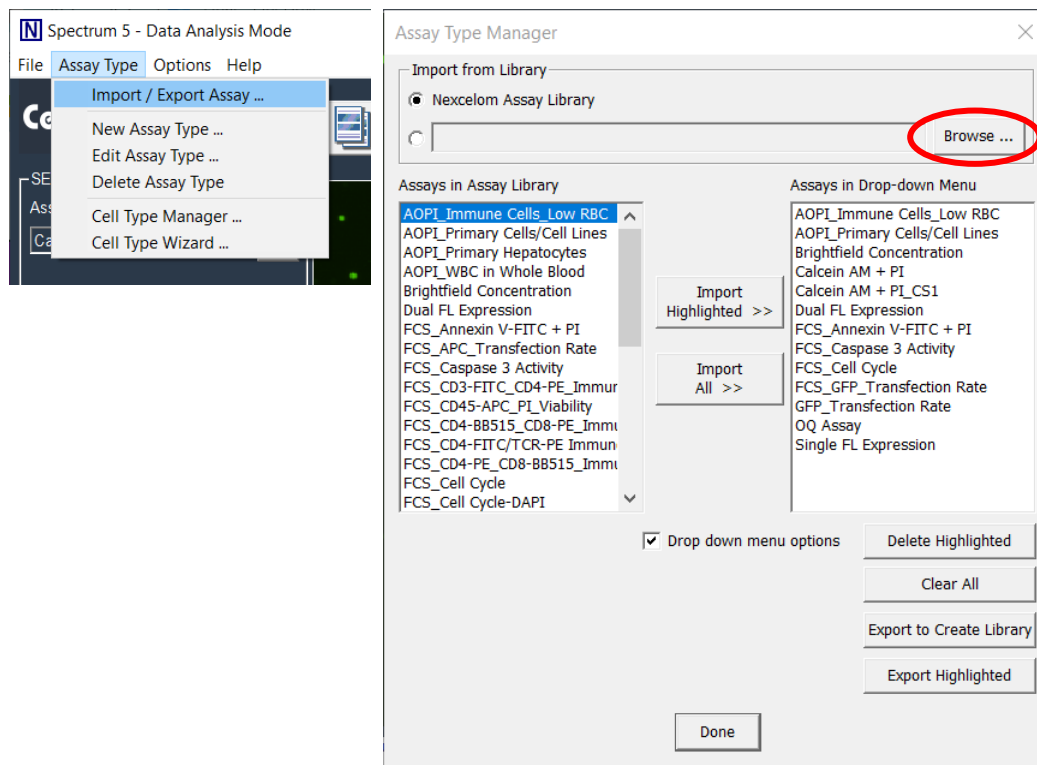
3. Highlight the cell type in the **Cell Types in Cell Library** and click **Import Highlighted**. For the Calcein AM/PI assay, the Calcein AM cell type should be imported.



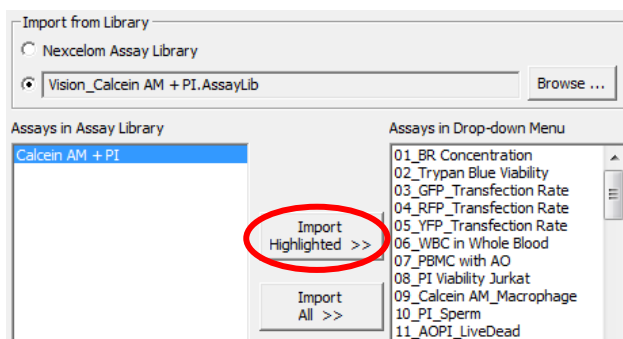
4. Repeat steps to import the PI cell type.

8.2 Importing an Assay

1. Locate and click on the “Assay Type” at the top of the screen. Followed by Import / Export Assay.
2. Once the Assay Type Manager appears, click on Browse, navigate to the desktop and locate the “Vision_Calcein AM.CellLib” file.



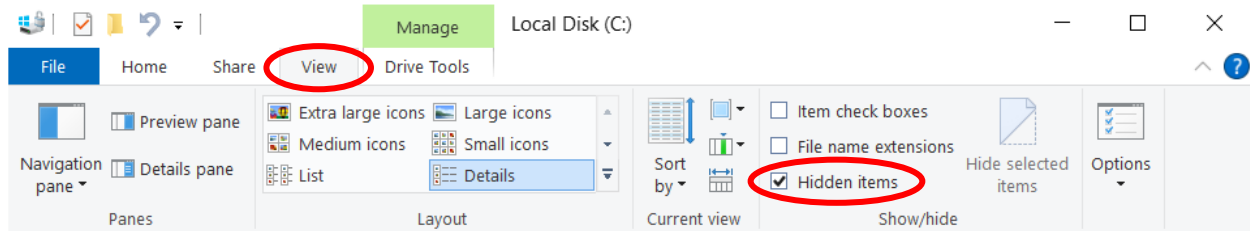
3. Highlight the assay in the **Assays in Assay Library** and click **Import Highlighted**. For the Calcein AM/PI assay, the Calcein AM +PI assay should be imported.
4. This assay will now be available from the dropdown menu.



5. The Calcein AM + PI_CS assay will now be available from the drop down menu.

8.3 Importing a Result or Print Template

1. From the File Explorer navigator open the **C: Drive** and locate the **ProgramData** folder.
 - 1.1. If the ProgramData folder is not listed, it may be hidden. To unhide a folder, click on the **View** Tab and ensure the **Hidden items** check box is selected.



2. Open the **ProgramData** folder.
3. Locate and open the **Nexcelom_Spectrum_v321** (or software equivalent) folder.
4. Open the **Template** folder.
5. Add the result template (Calcein AM and PI.rlt_tm) and/or print template (Calcein AM and PI.prn_tm) files into the Template folder. They will now be available to choose from when editing the assay.

9 Storage and Handling

Store the Propidium Iodide (Component A) between 2 and 8 °C and the Calcein AM (Component B) between -16 and -24 °C. Please consult the Safety Data Sheet for more safety information, found on www.revvity.com/cellcountingreagents.

10 Warranty

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

Warranty will be void if product is stored incorrectly, the recommended protocol is not followed, or the product is used for a different application.

11 Ordering Information / Support

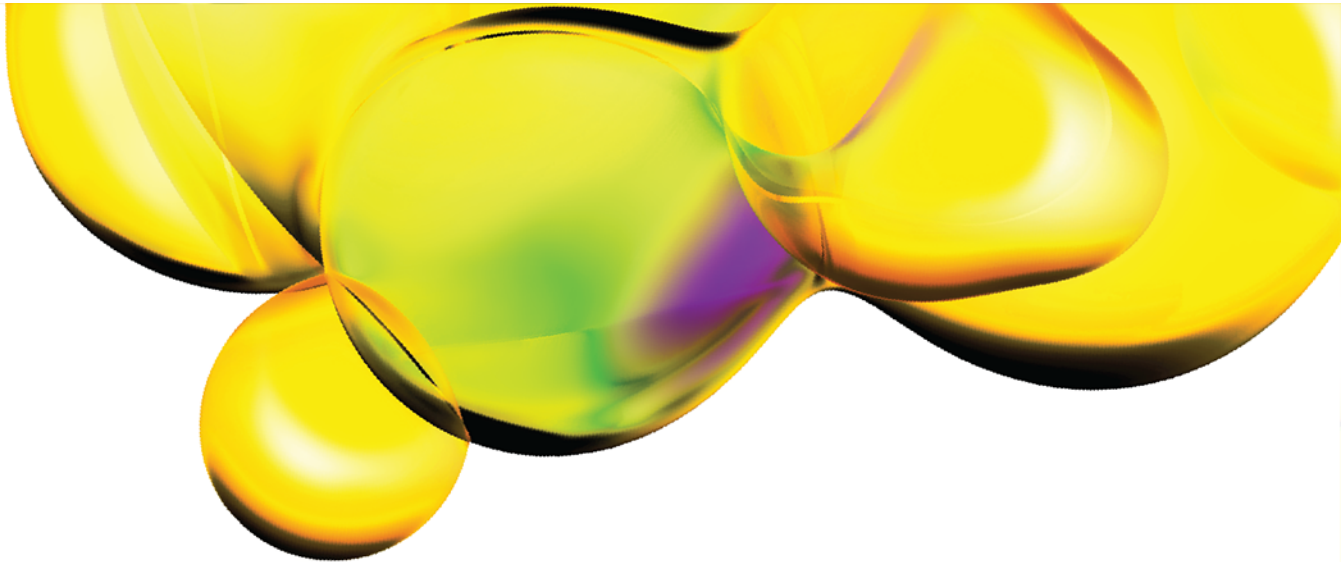
When ordering with a Purchase Order:

E-mail a copy of the order to Cellc-sales@revvity.com

For online orders, please visit:

<https://www.revvity.com/cellcountingreagents>

For support, e-mail USCAN.service@revvity.com



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