

## PRODUCT INSERT

Instrument Compatibility: Cellaca<sup>®</sup> PLX

# Cellaca<sup>®</sup> PLX, Hoechst / RubyDead Viability Kit

Part number:	CSK-A0030-1	CSK-A0030-2
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Test number:	25 Tests	100 Tests
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Storage: 4°C

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## 1. Introduction

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### 1.1. Description

Fluorescent proteins stained with viability dyes are designed for researchers interested in acquiring data on protein expression and viability, as each cell line derived sample can be unique. The Cellaca® PLX provides users with fluorescent and bright field images of their GFP or RFP cells, as well as dead (RubyDead) and total (Hoechst) dye stained cells. Data can be automatically exported from PLX Matrix software into FCS Express software templates with preset gates for rapid data analysis.

### 1.2. Kit contents

This kit assesses the viability of either GFP or RFP expressing cells on the Cellaca® PLX. For viability, dead cells are identified using the RubyDead dye, while total cells are stained with Hoechst. See table below for kit components.

Cellaca® PLX Assay	Reagents	Catalog Number	Number of Tests
PLX.5_FL Proteins__GFP + Hoechst + RubyDead	<b>RubyDead Dye</b> (Component A)	CSK-A0030-1	25
PLX.5_FL Proteins__RFP + Hoechst + RubyDead	<b>Hoechst 33342</b> (Component B)	CSK-A0030-2	100

### 1.3. Required Materials

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- Cellaca® PLX image cytometer (Revvity)
- Revvity-provided Laptop with Matrix 5.0 Software or above (pre-installed)
- FCS Express software (pre-installed on Revvity-provided laptop) with dongle/license
- Counting Plate (Cat. # CHM24-A100 or CHM24-B100) **OR** Cellaca® PLX Low Fluorescence Slides (Cat. # CHM2-ACR)
- Cellaca® PLX slide holder (if using slides)
- Reagents provided in kit CSK-A0030
- Microcentrifuge tubes
- Cell culture media
- 1X Phosphate Buffered Saline (PBS)
- RFP or GFP Cells

## 2. Staining Procedure for GFP or RFP cells with Hoechst and RubyDead

Cellaca® PLX Assay	Reagents	Catalog Number	Number of Tests
PLX.5_FL Proteins__GFP + Hoechst + RubyDead	<b>RubyDead Dye</b> (Component A)	CSK-A0030-1	25
PLX.5_FL Proteins__RFP + Hoechst + RubyDead	<b>Hoechst 33342</b> (Component B)	CSK-A0030-2	100

- For a single sample, prepare a microcentrifuge tube with  $1 \times 10^6$  GFP or RFP cells  
***NOTE 1:** For  $1 \times 10^6$  cells, take 1 mL of  $1 \times 10^6$  cells/mL*  
***NOTE 2:** For multiple samples, prepare corresponding tubes*
- Centrifuge cells at 1200 rpm for 5 minutes
- Remove supernatant from each tube avoiding cell pellets
- Resuspend each cell pellet in 100  $\mu$ L of cell culture media
- Dilute RubyDead Dye by adding 1  $\mu$ L of **RubyDead Dye** (Component A) to 1  $\mu$ L DMSO  
***NOTE 1:** 1:2 dilution for 100X working stock*  
***NOTE 2:** If staining 2-4 samples, prepare additional RubyDead Dye, according to the table below*

	2 samples	3 samples	4 samples
<b>RubyDead Dye</b> (Component A)	1.5 $\mu$ L	2 $\mu$ L	2.5 $\mu$ L
<b>DMSO</b>	1.5 $\mu$ L	2 $\mu$ L	2.5 $\mu$ L

- Dilute Hoechst 33342 by adding 1  $\mu$ L of **Hoechst 33342** (Component B) to 19  $\mu$ L 1X PBS  
***NOTE:** 1:20 dilution for 1 mM working stock*
- For staining cells in each tube, add the following, and mix well:
  - 1  $\mu$ L of RubyDead Dye working stock (diluted from step 5)
  - 1  $\mu$ L of Hoechst working stock (diluted from step 6)***NOTE:** If testing 2-4 samples, we recommend creating a master mix, according to the table below. After adding all components to form the master mix, add 2  $\mu$ L of the master mix stain to each tube with cells and mix well.*

	2 samples	3 samples	4 samples
<b>RubyDead Dye working stock</b> (Diluted from step 5)	2.2 $\mu$ L	3.3 $\mu$ L	4.4 $\mu$ L
<b>Hoechst working stock</b> (Diluted from step 6)	2.2 $\mu$ L	3.3 $\mu$ L	4.4 $\mu$ L

- Incubate tube(s) in the dark for 15 minutes at room temperature (25 °C)
- Mix samples thoroughly by pipetting up and down a few times

**10.** Load samples into consumable

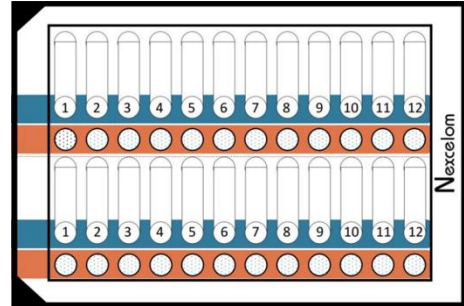
- **For Counting Plates**, load 50  $\mu$ L of each sample into a loading well
- **For Low Fluorescence Slides**, load 15  $\mu$ L of each sample into one side of the slide

***NOTE:** For additional samples or replicates from the same sample, load subsequent windows in slides*

**11.** If using slides, place into slide holder, with A at the top, as shown in the diagram

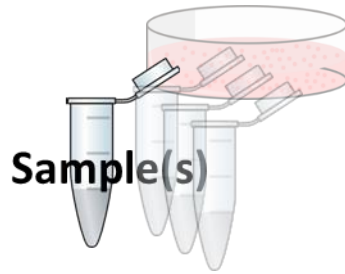
***NOTE:** Notched edge of the slide holder is the top left*

**12.** For slides or plates, proceed to section 4 for image and data acquisition



### 3. Expert User Quick Guide – GFP or RFP cells with Hoechst and RubyDead

Add  $1 \times 10^6$   
cells/tube



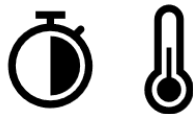
1200 rpm, 5 min



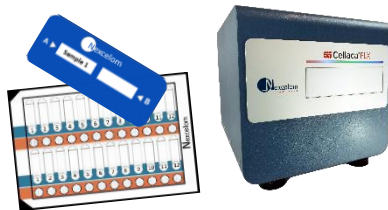
Remove supernatant  
Resuspend cell pellet  
in 100  $\mu$ L of cell  
culture media  
Add reagents\*



Incubate RT, 15 min



Load samples into  
plates or slides and  
image on Cellaca®  
PLX



\* Dilute **Hoechst** 1:20 in 1X PBS

\* Dilute **RubyDead** 1:2 in DMSO

\* For **each tube**:

	Samples			
	1	2	3	4
<b>RubyDead</b>	1 $\mu$ L	2.2 $\mu$ L	3.3 $\mu$ L	4.4 $\mu$ L
<b>Hoechst</b>	1 $\mu$ L	2.2 $\mu$ L	3.3 $\mu$ L	4.4 $\mu$ L

Add 2  $\mu$ L of the master mix to each tube

## 4. Cellaca® PLX Image and Data Acquisition

### 4.1. Initiate software and load samples

- 4.1.1. Start the **Matrix** software by double-clicking the icon on the desktop of the operating computer
- 4.1.2. Software will direct you to the **Acquire, Setup** tab by default
- 4.1.3. Click **Eject** to open the instrument stage  
*NOTE: Button located at the top of the Acquire tab*
- 4.1.4. Place the counting plate or the slide holder containing slide(s) into the ejected stage  
*NOTE: Align the notched edge of the counting plate or slide holder in the upper left corner*
- 4.1.5. Click the **Load** button to retract the instrument stage



### 4.2. Assay Selection

- 4.2.1. In **Setup Details**, type in a **Plate Name**
- 4.2.2. **Select Assay** from the dropdown according to the cell line (GFP or RFP) and consumable being used

Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead	▼	View
Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead - slide	▼	View
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead	▼	View
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead - slide	▼	View

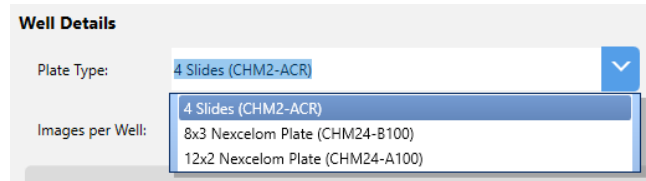
- 4.2.3. To edit or review assay settings, click the blue **View** tab to the right of the assay selection

*NOTE: See Assay Settings, Cell Type Parameters, and Auto Export Data and Images sections in the Appendix for detailed information regarding assay, cell parameters, and report/export information, respectively*

### 4.3. Well Details and Assign Well Names


#### 4.3.1. In **Well Details**:

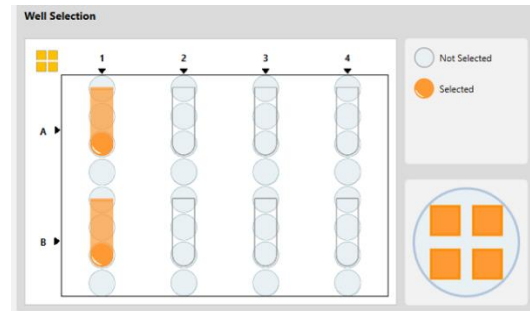
- 4.3.1.1. Select the **Plate Type** from the dropdown according to the consumable being used



- 4.3.2. In **Well Selection**, select the well(s) to be imaged

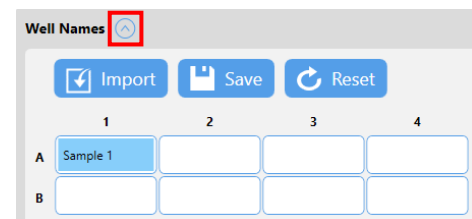
**NOTE 1:** Selected samples will turn orange.

**NOTE 2:** To select or clear multiple wells, click a well and hold/drag your mouse to encompass other wells. To select or clear all wells, click the  button



- 4.3.3. To assign **Well Names**, click the downward facing arrow

- 4.3.3.1. Type in the names of the well(s) / sample(s)

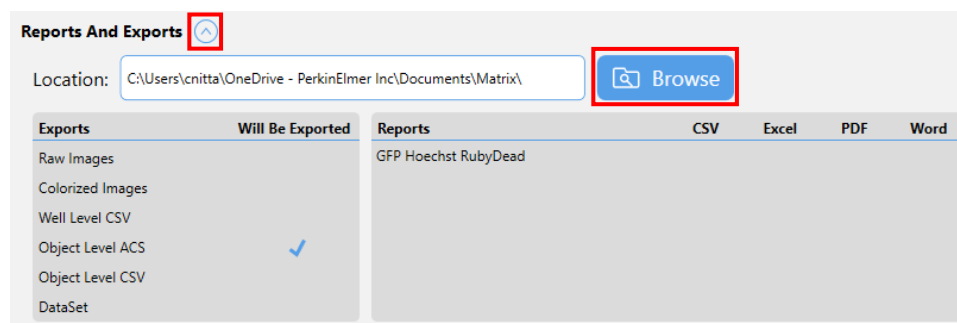


### 4.4. Reports and Exports

- 4.4.1. Click the downward facing arrow to open the reports and exports details

- 4.4.2. In **Location**, click on the browse button to select or create an export location.

**NOTE:** Images and data selected to be exported will have a blue checkmark

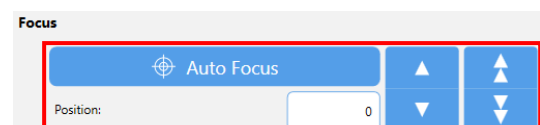


### 4.5. Preview Samples

- 4.5.1. Click the **Preview** button to view the sample

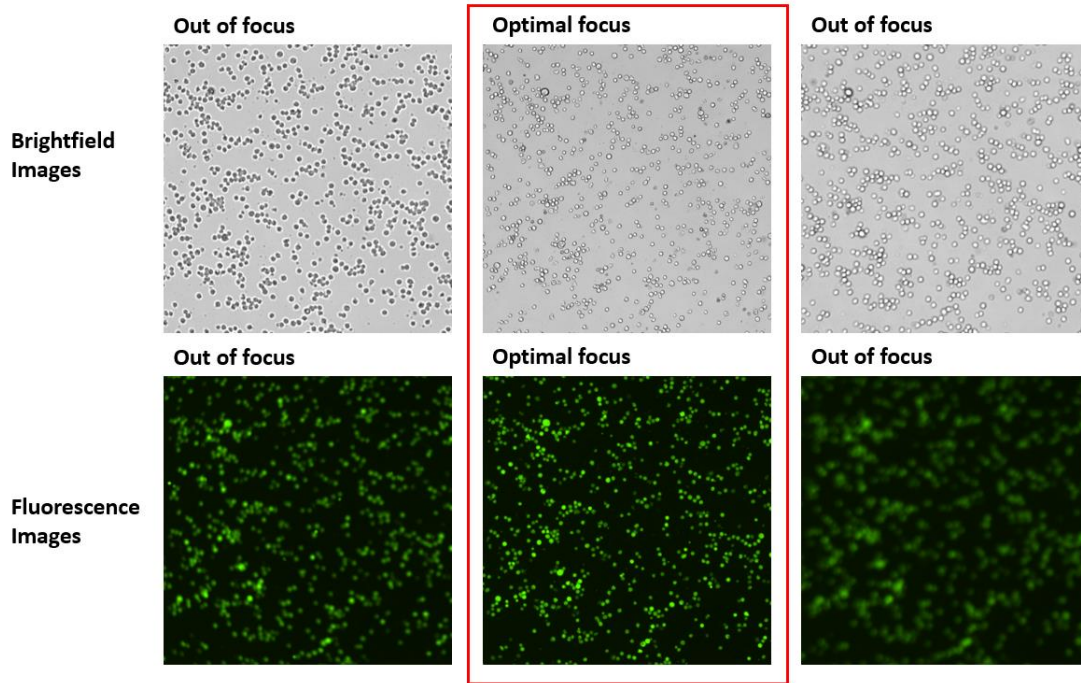


- 4.5.2. In **Focus**, click **Auto Focus** to focus the sample in Brightfield for Channel 1





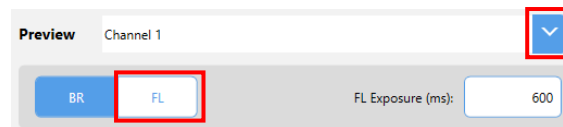
**NOTE:** If needed, manual focusing can be done using **double arrows** for coarse and **single arrow** for fine adjustments



4.5.3. Once the sample is focused, click the **FL** button to preview the Channel 1 fluorescence

4.5.3.1. Adjust exposure times as needed

**NOTE:** See *Recommended GFP/RFP and Viability Dyes Exposure Times and Filter Pairs in the Appendix*



4.5.4. Select subsequent fluorescence channels using the **Preview** dropdown menu

4.5.5. Click the **FL** button to preview the fluorescence in each channel and adjust exposure times as needed

4.5.6. Click the **Count** button when ready to acquire and analyze samples

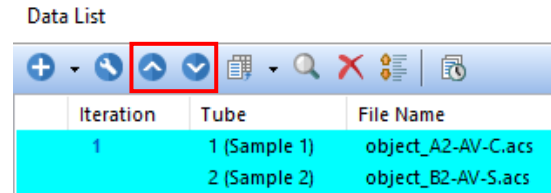


## 4.6. FCS Express

4.6.1. FCS Express will automatically initialize and populate with data generated from this scan

4.6.2. In the **Data List**, you can adjust the order of where your sample data appears in the template by using the up and down arrows to move them to the correct location

Data List



Iteration	Tube	File Name
1	1 (Sample 1)	object_A2-AV-C.acs
2	2 (Sample 2)	object_B2-AV-S.acs

## 5. Additional Resources

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### 5.1. Storage / Safety

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Store each product at 4 °C, protected from light. Please consult the Safety Data Sheet for more safety information, found on [www.revvity.com/cellcountingreagents](http://www.revvity.com/cellcountingreagents).

### 5.2. Warranty

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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.

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

### 5.3. Ordering Information / Support

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When ordering with a Purchase Order:

E-mail a copy of the order to [Cellc-sales@revvity.com](mailto:Cellc-sales@revvity.com)

For online orders, please visit:

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

For support, e-mail [Cellc-support@revvity.com](mailto:Cellc-support@revvity.com)

## 6. Appendix

### 6.1. Assay Settings


6.1.1. To edit or review assay settings, click the **View** button next to the selected assay, according to the “Plate type” being used

Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead	▼	 View
Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead - slide	▼	 View
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead	▼	 View
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead - slide	▼	 View


6.1.2. Click the downward facing arrow in **Imaging and Analysis** to edit or review settings

Imaging and Analysis 

**NOTE:** Below are the default assay settings for the Cellaca® PLX, Hoechst / RubyDead Viability Kit when using the CHM24-A100, CHM24-B100, or CHM2-ACR consumables

Imaging and Analysis 

**Imaging Mode**

BR  BR/FL Number of Channels: 3 

Two-Color Fluorescence w/ Brightfield Imaging: Accurate Cell Counts / Viability / Dual FL Expression

**Analysis Mode**

Expression

Dual Fluorescence Analysis For Samples Containing Two FL Stains

Mask:  BR  FL

Use the channel selected as the FL mask channel


Expand (µm):

Amount, in microns, to expand or contract the found mask object which is used to collect FL intensity measurements in all channels

**Focusing Mode**

Focus Map  Auto Focus 1st Well  Auto Focus All Wells

Auto Focus Is Applied To Every Well For Best Focus/Image Quality

Auto Focus Image:  

**Dilution**

Dilution Factor For General Assay As Indicated By Sample Preparation Protocol

**NOTE:** Below are the default Imaging Parameters for each channel in the Cellaca® PLX, Hoechst / RubyDead Viability Kit with GFP cells when using the CHM24-A100 or CHM24-B100 consumable (counting plate)

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b>	<b>Fluorescence</b>	<b>Filters</b>
Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/>	Fluorophore Name: <input type="text" value="Hoechst"/>	Excitation: <input checked="" type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input type="checkbox"/> 620
Custom Exposure Factor: <input type="text" value="1.0"/>	Exposure (ms): <input type="text" value="300"/>	Emission: <input checked="" type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692

Cell Type Parameters

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b>	<b>Fluorescence</b>	<b>Filters</b>
Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/>	Fluorophore Name: <input type="text" value="GFP"/>	Excitation: <input type="checkbox"/> 365 <input checked="" type="checkbox"/> 470 <input type="checkbox"/> 531 <input type="checkbox"/> 620
Custom Exposure Factor: <input type="text" value="1.0"/>	Exposure (ms): <input type="text" value="75"/>	Emission: <input type="checkbox"/> 452 <input checked="" type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b>	<b>Fluorescence</b>	<b>Filters</b>
Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/>	Fluorophore Name: <input type="text" value="RubyDead"/>	Excitation: <input type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input checked="" type="checkbox"/> 620
Custom Exposure Factor: <input type="text" value="1.0"/>	Exposure (ms): <input type="text" value="500"/>	Emission: <input type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input checked="" type="checkbox"/> 692

**NOTE:** Below are the default Imaging Parameters for each channel in the Cellaca® PLX, Hoechst / RubyDead Viability Kit with GFP cells when using the CHM2-ACR consumable (low fluorescence slide)

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b> Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/> Custom Exposure Factor: <input type="text" value="1.0"/>	<b>Fluorescence</b> Fluorophore Name: <input type="text" value="Hoechst"/> Exposure (ms): <input type="text" value="600"/>	<b>Filters</b> Excitation: <input checked="" type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input type="checkbox"/> 620 Emission: <input checked="" type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692
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Cell Type Parameters

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b> Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/> Custom Exposure Factor: <input type="text" value="1.0"/>	<b>Fluorescence</b> Fluorophore Name: <input type="text" value="GFP"/> Exposure (ms): <input type="text" value="75"/>	<b>Filters</b> Excitation: <input type="checkbox"/> 365 <input checked="" type="checkbox"/> 470 <input type="checkbox"/> 531 <input type="checkbox"/> 620 Emission: <input type="checkbox"/> 452 <input checked="" type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692
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Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b> Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/> Custom Exposure Factor: <input type="text" value="1.0"/>	<b>Fluorescence</b> Fluorophore Name: <input type="text" value="RubyDead"/> Exposure (ms): <input type="text" value="1200"/>	<b>Filters</b> Excitation: <input type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input checked="" type="checkbox"/> 620 Emission: <input type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input checked="" type="checkbox"/> 692
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**NOTE:** Below are the default Imaging Parameters for each channel in the Cellaca® PLX, Hoechst / RubyDead Viability Kit with RFP cells when using the CHM24-A100 or CHM24-B100 consumable (counting plate)

Channel 1
Channel 2
Channel 3

**Imaging Parameters**

<p><b>Brightfield</b></p> <p>Use Custom Exposure: <span style="margin-left: 20px;"><input type="button" value="No"/></span> <span style="margin-left: 20px;"><input type="button" value="Yes"/></span></p> <p>Custom Exposure Factor: <input style="width: 50px;" type="text" value="1.0"/></p>	<p><b>Fluorescence</b></p> <p>Fluorophore Name: <input style="width: 80%;" type="text" value="Hoechst"/></p> <p>Exposure (ms): <input style="width: 50px;" type="text" value="300"/></p>	<p><b>Filters</b></p> <p>Excitation: <input checked="" type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input type="checkbox"/> 620</p> <p>Emission: <input checked="" type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692</p>
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**Cell Type Parameters** FL Proteins\_RFP + Hoechst + RubyDead

Channel 1
Channel 2
Channel 3

**Imaging Parameters**

<p><b>Brightfield</b></p> <p>Use Custom Exposure: <span style="margin-left: 20px;"><input type="button" value="No"/></span> <span style="margin-left: 20px;"><input type="button" value="Yes"/></span></p> <p>Custom Exposure Factor: <input style="width: 50px;" type="text" value="1.0"/></p>	<p><b>Fluorescence</b></p> <p>Fluorophore Name: <input style="width: 80%;" type="text" value="RFP"/></p> <p>Exposure (ms): <input style="width: 50px;" type="text" value="2500"/></p>	<p><b>Filters</b></p> <p>Excitation: <input type="checkbox"/> 365 <input type="checkbox"/> 470 <input checked="" type="checkbox"/> 531 <input type="checkbox"/> 620</p> <p>Emission: <input type="checkbox"/> 452 <input type="checkbox"/> 534 <input checked="" type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692</p>
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Channel 1
Channel 2
Channel 3

**Imaging Parameters**

<p><b>Brightfield</b></p> <p>Use Custom Exposure: <span style="margin-left: 20px;"><input type="button" value="No"/></span> <span style="margin-left: 20px;"><input type="button" value="Yes"/></span></p> <p>Custom Exposure Factor: <input style="width: 50px;" type="text" value="1.0"/></p>	<p><b>Fluorescence</b></p> <p>Fluorophore Name: <input style="width: 80%;" type="text" value="RubyDead"/></p> <p>Exposure (ms): <input style="width: 50px;" type="text" value="500"/></p>	<p><b>Filters</b></p> <p>Excitation: <input type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input checked="" type="checkbox"/> 620</p> <p>Emission: <input type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input checked="" type="checkbox"/> 692</p>
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**NOTE:** Below are the default Imaging Parameters for each channel in the Cellaca® PLX, Hoechst / RubyDead Viability Kit with RFP cells when using the CHM2-ACR consumable (low fluorescence slide)

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b> Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/> Custom Exposure Factor: <input type="text" value="1.0"/>	<b>Fluorescence</b> Fluorophore Name: <input type="text" value="Hoechst"/> Exposure (ms): <input type="text" value="600"/>	<b>Filters</b> Excitation: <input checked="" type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input type="checkbox"/> 620 Emission: <input checked="" type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692
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Cell Type Parameters

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Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b> Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/> Custom Exposure Factor: <input type="text" value="1.0"/>	<b>Fluorescence</b> Fluorophore Name: <input type="text" value="RFP"/> Exposure (ms): <input type="text" value="3500"/>	<b>Filters</b> Excitation: <input type="checkbox"/> 365 <input type="checkbox"/> 470 <input checked="" type="checkbox"/> 531 <input type="checkbox"/> 620 Emission: <input type="checkbox"/> 452 <input type="checkbox"/> 534 <input checked="" type="checkbox"/> 605 <input type="checkbox"/> 655 <input type="checkbox"/> 692
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Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<b>Brightfield</b> Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/> Custom Exposure Factor: <input type="text" value="1.0"/>	<b>Fluorescence</b> Fluorophore Name: <input type="text" value="RubyDead"/> Exposure (ms): <input type="text" value="1200"/>	<b>Filters</b> Excitation: <input type="checkbox"/> 365 <input type="checkbox"/> 470 <input type="checkbox"/> 531 <input checked="" type="checkbox"/> 620 Emission: <input type="checkbox"/> 452 <input type="checkbox"/> 534 <input type="checkbox"/> 605 <input type="checkbox"/> 655 <input checked="" type="checkbox"/> 692
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## 6.2. Cell Type Parameters

6.2.1 To edit or review assay settings, click the **View** button next to the selected assay

Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead	▼	<b>View</b>
Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead - slide	▼	<b>View</b>
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead	▼	<b>View</b>
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead - slide	▼	<b>View</b>

6.2.2 Click the downward facing arrow in **Imaging and Analysis** to edit or review settings

**Imaging and Analysis** ▼

6.2.3 In **Imaging Parameters**, ensure Channel 1 is selected to view **Cell Type Parameters**

6.2.4 Ensure that the **Cell Type Parameter** selected corresponds to the kit being used

<b>Cell Type Parameters</b>	FL Proteins_RFP + Hoechst + RubyDead	▼	<b>View</b>
<b>Cell Type Parameters</b>	FL Proteins_GFP + Hoechst + RubyDead	▼	<b>View</b>

6.2.5 To edit or review Cell Type Parameters, click the **View** button

**NOTE:** Below are the default Cell Parameters for the Cellaca® PLX, Hoechst / RubyDead Viability Kit with GFP cells

**Brightfield Parameters**

<b>Cell Attributes</b>	<b>Decustering</b>	<b>Trypan Blue</b>
Cell Diameter (µm): 5.0 to 50.0	<input type="radio"/> No <input checked="" type="radio"/> Yes	Dead Cell Diameter (µm): 4.0 to 50.0
Roundness: 0.05	Edge Factor: 0.4	Sensitivity: 1.0
Contrast Enhancement: 0.30	Threshold Factor: 1.0	Uniformity: 150
	Background Adjustment: 0.4	Very Dim Dead Cells: <input checked="" type="radio"/> No <input type="radio"/> Yes
		Contrast Enhancement: 0.60

**Fluorescence Parameters**

<b>Cell Attributes</b>	<b>Thresholding</b>
Cell Diameter (µm): 3.0 to 40.0	<input checked="" type="radio"/> Manual <input type="radio"/> Auto
Normalize intensity for cell size: <input type="radio"/> No <input checked="" type="radio"/> Yes	% of Image Range to Count: 10
Non-Uniform Cells: <input type="radio"/> No <input checked="" type="radio"/> Yes	Threshold Factor: 1.0
Roundness: 0.10	
Do Not Count Free Nuclei: <input type="radio"/> No <input checked="" type="radio"/> Yes	
Advanced BR/F Mode: <input type="radio"/> No <input checked="" type="radio"/> Yes	

**NOTE:** Below are the default Cell Parameters for the Cellaca® PLX, Hoechst / RubyDead Viability Kit with RFP cells

**Brightfield Parameters**

<b>Cell Attributes</b>	<b>Decustering</b>	<b>Trypan Blue</b>
Cell Diameter (µm): 5.0 to 50.0	Edge Factor: 0.7	Dead Cell Diameter (µm): 4.0 to 50.0
Roundness: 0.05	Threshold Factor: 1.0	Sensitivity: 1.0
Contrast Enhancement: 0.80	Background Adjustment: 1.0	Uniformity: 150
		Very Dim Dead Cells: No Yes
		Contrast Enhancement: 0.60

**Fluorescence Parameters**

<b>Cell Attributes</b>	<b>Thresholding</b>
Cell Diameter (µm): 4.0 to 50.0	Manual Auto
Normalize intensity for cell size: No Yes	% of Image Range to Count: 10
Non-Uniform Cells: No Yes	Threshold Factor: 1.0
Roundness: 0.10	
Do Not Count Free Nuclei: No Yes	
Advanced BR/F Mode: No Yes	

### 6.3. Auto Export Data and Images

6.3.1 To edit or review assay settings, click the **View** button next to the selected assay

Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead	View
Select Assay:	PLX.5_FL Proteins_GFP + Hoechst + RubyDead - slide	View
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead	View
Select Assay:	PLX.5_FL Proteins_RFP + Hoechst + RubyDead - slide	View

6.3.2 Click the downward facing arrow in **Reports and Exports** to edit or review settings

6.3.3 In **Display**, ensure the correct display is selected according to the cell line being used

The screenshot shows the 'Reports and Exports' configuration panel. At the top, the 'Display' dropdown menu is set to 'GFP Hoechst RubyDead'. Below this, the 'Exports' section has three sub-sections: 'Images' with 'Raw Images' and 'Colorized Images' buttons; 'Data' with 'Well Level CSV', 'Object Level CSV', and 'Object Level ACS' buttons, where 'Object Level ACS' is checked; and 'Object Level ACS Options' with 'Use Template' checked. A dropdown menu below 'Object Level ACS Options' is set to 'FLprot+viab\_GFP + Hoechst + RubyDead', and the 'Auto Open' button is checked. At the bottom, the 'Archive' section has a 'Data Set' button.

6.3.4 In **Exports**, select what you would like to be automatically exported after each scan when using this assay

6.3.4.1 For automatic export to FCS Express for viability analysis, select **Object Level ACS**, ensure **Use Template** is selected, and that the appropriate Template is selected, with the **Auto Open** button selected

#### 6.4. Recommended GFP/RFP and Viability Dyes Exposure Times and Filter Pairs

Recommended imaging parameters and exposure times (with ranges) for Hoechst, GFP or RFP, and RubyDead on Cellaca® PLX. Exposure times may require optimization due to the individuality of each cell line.

*With GFP cells when using the CHM24-A100 or CHM24-B100 consumable (counting plate)*

<b>Cellaca® PLX Excitation / Emission</b>	<b>Illumination</b>	<b>Reagent</b>	<b>Assay Default Exposure Time (ms) (Recommended range)</b>
365 / 452	Blue	Hoechst 33342	<b>300</b> (250 – 600)
470 / 534	Green	GFP	<b>75</b> (50 – 150)
620 / 692	Far Red	RubyDead	<b>500</b> (400 – 800)

*With GFP cells when using the CHM2-ACR consumable (Low fluorescence slide)*

<b>Cellaca® PLX Excitation / Emission</b>	<b>Illumination</b>	<b>Reagent</b>	<b>Assay Default Exposure Time (ms) (Recommended range)</b>
365 / 452	Blue	Hoechst 33342	<b>600</b> (400 – 800)
470 / 534	Green	GFP	<b>75</b> (50 – 150)
620 / 692	Far Red	RubyDead	<b>1,200</b> (1,000 – 1,500)

*With RFP cells when using the CHM24-A100 or CHM24-B100 consumable (counting plate)*

<b>Cellaca® PLX Excitation / Emission</b>	<b>Illumination</b>	<b>Reagent</b>	<b>Assay Default Exposure Time (ms) (Recommended range)</b>
365 / 452	Blue	Hoechst 33342	<b>300</b> (250 – 600)
531 / 605	Orange	RFP	<b>2,500</b> (2,000 – 3,500)
620 / 692	Far Red	RubyDead	<b>500</b> (400 – 800)



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