

## PRODUCT INSERT

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

# Cellaca<sup>®</sup> PLX, anti-human CD3 APC / CD8 PE Total Cell Kit

Part number:	CSK-A0013-1	CSK-A0013-2
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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

CD3/CD8 surface marker reagents with a total dye (Hoechst) are designed for researchers interested in acquiring data on two surface marker populations, as each patient and cell line derived sample can be unique. The Cellaca® PLX provides users with fluorescent and bright field images of their Hoechst and CD3/CD8 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 CD3/CD8 populations with Hoechst as a total dye on the Cellaca® PLX. The anti-human CD3 antibody is conjugated with APC and the anti-human CD8 antibody is conjugated with PE. See table below for kit components and corresponding surface markers with their respective isotype controls.

Cellaca® PLX Assay	Reagents	Catalog Number	Number of Tests
PLX.5_2SM+Total__ CD3-APC + CD8-PE + Hoechst	<b>APC anti-human CD3 (HIT3α)</b> (Component A)	CSK-A0013-1	25
	<b>PE anti-human CD8 (RPA-T8)</b> (Component B)		
	<b>APC Mouse IgG2a Isotype</b> (Component C)	CSK-A0013-2	100
	<b>PE Mouse IgG1 Isotype</b> (Component D)		
	<b>Hoechst 33342</b> (Component E)		

### 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
- Cellaca® PLX Low Fluorescence Slides (Cat. # CHM2-ACR)
- Cellaca® PLX slide holder
- Reagents provided in kit CSK-A0013
- 1X Phosphate Buffered Saline (PBS)
- Microcentrifuge tubes
- Cell culture media
- Cells or PBMC's

## 2. Staining Procedure for CD3 APC / CD8 PE with Hoechst

Cellaca® PLX Assay	Reagents	Catalog Number	Number of Tests
PLX.5_2SM+Total__ CD3-APC + CD8-PE + Hoechst	<b>APC anti-human CD3 (HIT3α)</b> (Component A)	CSK-A0013-1	25
	<b>PE anti-human CD8 (RPA-T8)</b> (Component B)		
	<b>APC Mouse IgG2a Isotype</b> (Component C)	CSK-A0013-2	100
	<b>PE Mouse IgG1 Isotype</b> (Component D)		
	<b>Hoechst 33342</b> (Component E)		

### For each sample:

- For a single sample, prepare 2 microcentrifuge tubes with  $1 \times 10^6$  PBMCs/cells each  
**NOTE 1:** For  $1 \times 10^6$  cells, take 1 mL of  $1 \times 10^6$  cells/mL  
**NOTE 2:** For multiple samples, prepare 2 tubes each
- Label tubes, accordingly, one for staining with antibodies (**Ab**) and one for isotype control (**Ctrl**) staining for each distinct sample
- Centrifuge cells at 1200 rpm for 5 minutes
- Remove supernatant from all tubes avoiding cell pellets
- Dilute Hoechst 33342 by adding 1  $\mu$ L of **Hoechst 33342** (Component E) to 19  $\mu$ L 1X PBS  
**NOTE:** 1:20 dilution for 1 mM working stock
- Resuspend the cell pellets from all tubes in 90  $\mu$ L of cell culture media  
**NOTE:** Staining with PBS results in dimmer signal
- For staining cells in **Ab tubes**, add the following, and mix well:
  - 5  $\mu$ L of **CD3 APC** (Component A)
  - 5  $\mu$ L of **CD8 PE** (Component B)
  - 1  $\mu$ L of Hoechst 33342 (diluted from step 5)

**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 10.5  $\mu$ L of the master mix stain to each **Ab tube** and mix well.

	2 samples	3 samples	4 samples
<b>CD3 APC</b> (Component A)	10 $\mu$ L	15 $\mu$ L	20 $\mu$ L
<b>CD8 PE</b> (Component B)	10 $\mu$ L	15 $\mu$ L	20 $\mu$ L
<b>Hoechst working stock</b> (Diluted from step 5)	2 $\mu$ L	3 $\mu$ L	4 $\mu$ L

8. For staining cells in **Ctrl tubes**, add the following, and mix well:

- 1.2 µL of **IgG2a APC** (Component C)
- 1.2 µL of **IgG1 PE** (Component D)
- 1 µL of Hoechst 33342 (diluted from step 5)

**NOTE:** If testing 2-4 samples, we recommend creating an isotype control master mix, according to the table below. After adding all components to form the isotype control master mix, add 3.3 µL of the isotype control master mix stain to each **Ctrl tube** and mix well.

	2 samples	3 samples	4 samples
<b>IgG2a APC</b> (Component C)	2.5 µL	3.7 µL	5 µL
<b>IgG1 PE</b> (Component D)	2.5 µL	3.7 µL	5 µL
<b>Hoechst working stock</b> (Diluted from step 5)	2 µL	3 µL	4 µL

9. Incubate all tubes in the dark for 10 minutes at 37 °C

10. To each tube, add 200 µL of 1X PBS and mix well

11. Centrifuge cells at 1200 rpm for 5 minutes

12. Remove supernatant from each tube avoiding cell pellets

13. Resuspend each cell pellet in 100 µL of cell culture media

**NOTE:** Resuspension in 1X PBS results in dimmer signal

14. Mix samples thoroughly by pipetting up and down a few times

15. Load 15 µL of sample from **Ab tube** into side A of the slide

**NOTE 1:** Loading samples in wrong side results in incorrect sample output in FCS Express

**NOTE 2:** Repeat for any additional samples prepared

16. Load 15 µL of sample from **Ctrl tube** into side B of the slide

**NOTE:** Repeat for any additional samples prepared

17. To image replicates from the same sample, load another slide following steps 15 and 16

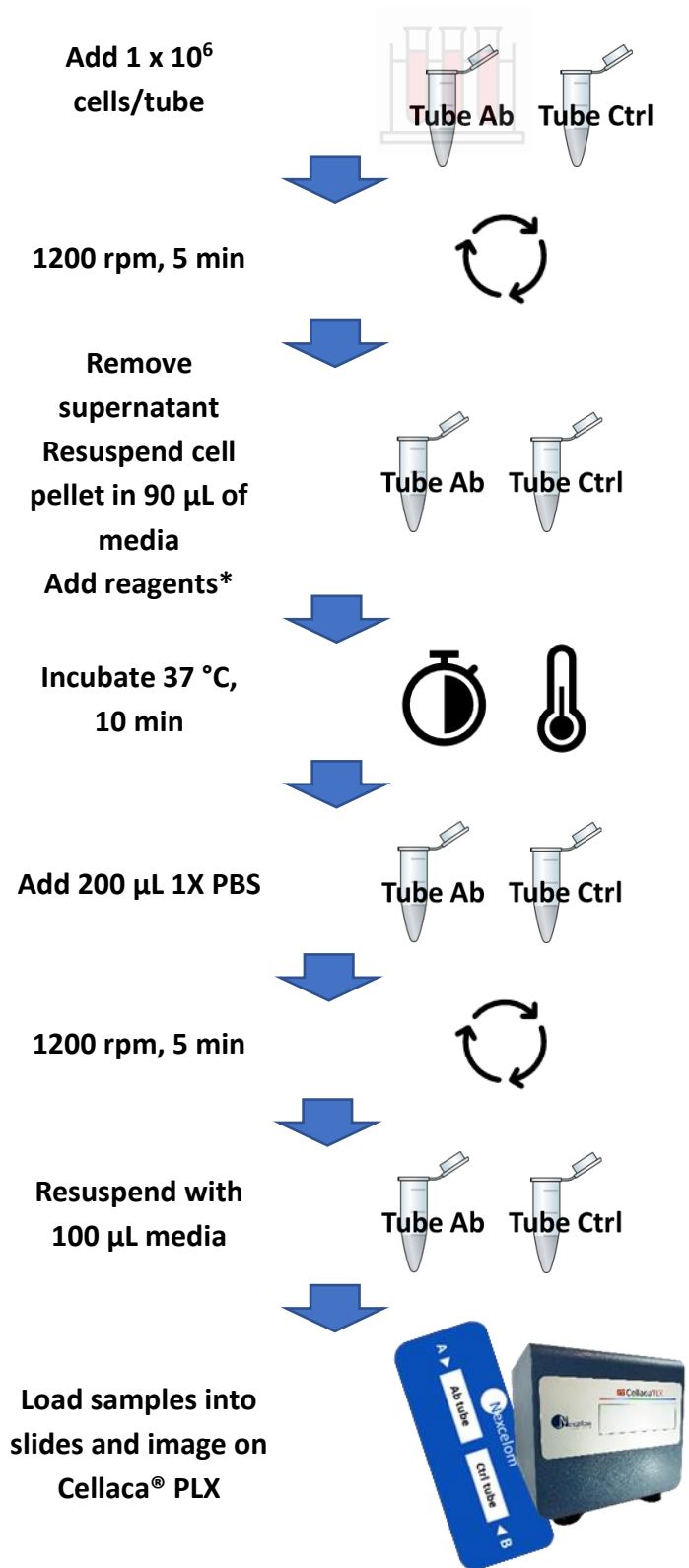
18. Place slides into slide holder, with side A at the top, as shown in the diagram

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

19. Proceed to section 4 for image and data acquisition



### 3. Expert User Quick Guide – CD3 APC / CD8 PE with Hoechst



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

\* For **Ab tubes:**

	Samples			
	1	2	3	4
<b>CD3 APC</b>	5 µL	10 µL	15 µL	20 µL
<b>CD8 PE</b>	5 µL	10 µL	15 µL	20 µL
<b>Hoechst</b>	1 µL	2 µL	3 µL	4 µL

Add 10.5 µL of the master mix to each tube

\* For **Ctrl tubes:**

	Samples			
	1	2	3	4
<b>IgG2a APC</b>	1.2 µL	2.5 µL	3.7 µL	5 µL
<b>IgG1 PE</b>	1.2 µL	2.5 µL	3.7 µL	5 µL
<b>Hoechst</b>	1 µL	2 µL	3 µL	4 µL

Add 3.3 µL 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 slide holder containing slide(s) into the ejected stage  
*NOTE: Align the notched edge of the 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

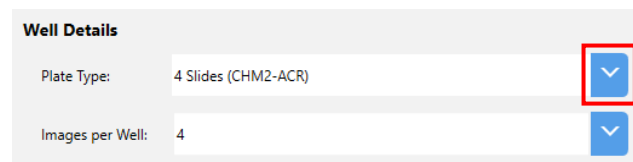


- 4.2.3. To edit or review assay settings, click the blue **View** tab to the right of the assay selection

*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

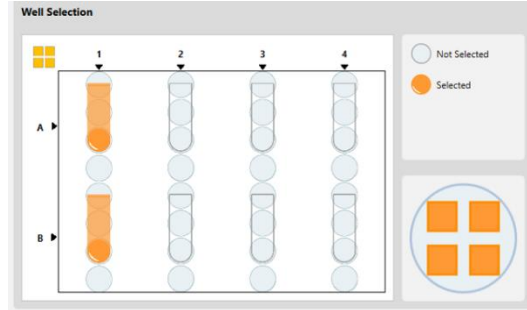
- 4.3.1. In **Well Details**:
  - 4.3.1.1. Select "4 Slides (CHM2-ACR)" as the **Plate Type**



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 well/sample name(s)

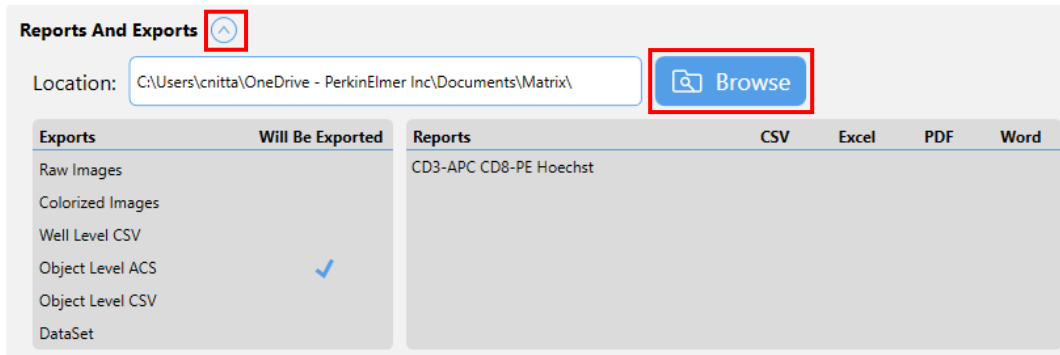


## 4.4. Reports and Exports

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

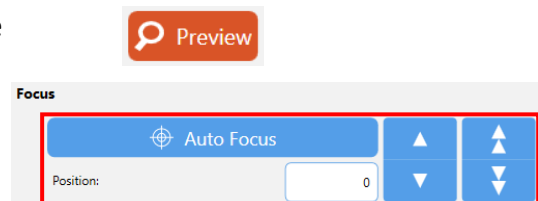


## 4.5. Preview Samples

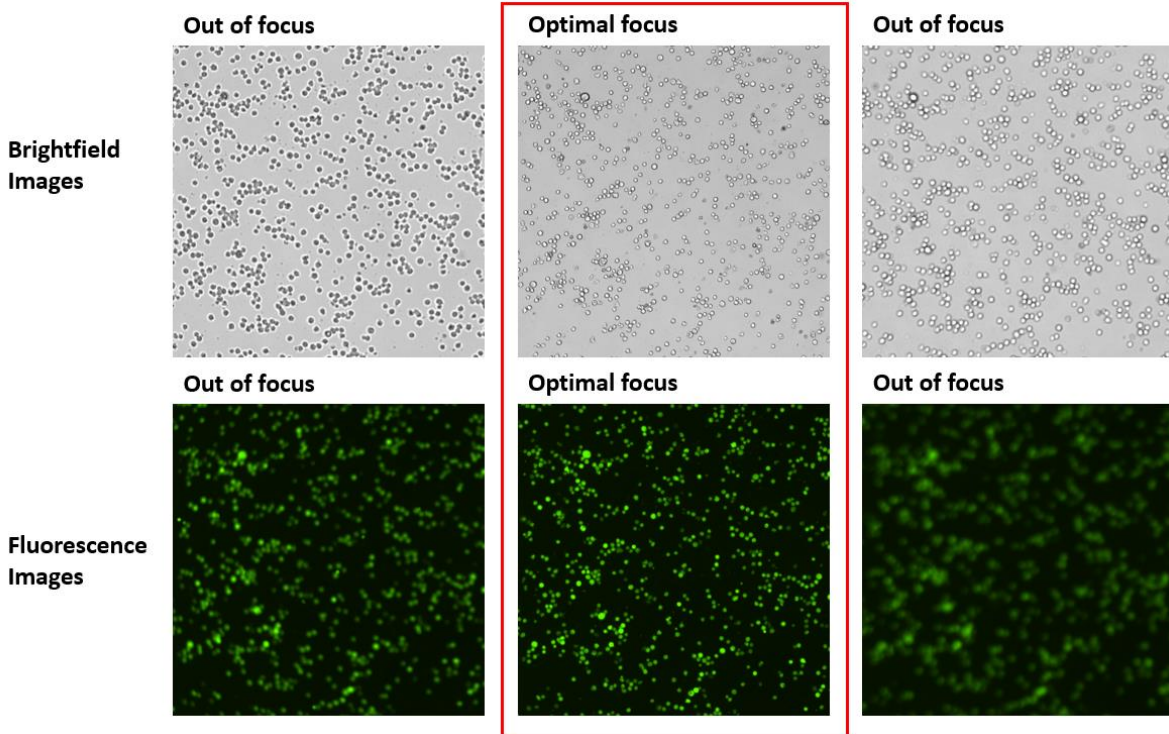
4.5.1. Click the **Preview** button to view the sample

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



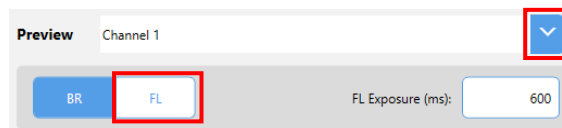




4.5.3. Once the sample is focused, click the **FL** button to preview Channel 1 fluorescence

4.5.3.1. Adjust exposure times as needed

**NOTE:** See *Recommended Surface Marker and Total Dye 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, confirm that your samples in the File Name column are in the correct order according to the Tube column (Ex: Sample 1 and Sample 1 Isotype as object\_A1.acs and object\_B1.acs, respectively)

**NOTE 1:** *If samples are not in the correct order, use the up and down arrows to move them to the correct location.*

**NOTE 2:** *If samples are not in the correct order data will not be accurate.*

Data List



Iteration	Tube	File Name
1	1 (Sample 1)	object_A1.acs
	2 (Sample 1 isotype)	object_B1.acs
	3 (Sample 2)	object_A2.acs
	4 (Sample 2 isotype)	object_B2.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



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



**NOTE:** Below are the default assay settings for the Cellaca® PLX, anti-human CD3 APC / CD8 PE Total Cell Kit

**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, anti-human CD3 APC / CD8 PE Total Cell Kit

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<p><b>Brightfield</b></p> <p>Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/></p> <p>Custom Exposure Factor: <input type="text" value="1.0"/></p>	<p><b>Fluorescence</b></p> <p>Fluorophore Name: <input type="text" value="Hoechst"/></p> <p>Exposure (ms): <input type="text" value="600"/></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** 2SM+Total\_CD3-APC + CD8-PE + Hoechst

Channel 1 Channel 2 Channel 3

**Imaging Parameters**

<p><b>Brightfield</b></p> <p>Use Custom Exposure: <input type="button" value="No"/> <input type="button" value="Yes"/></p> <p>Custom Exposure Factor: <input type="text" value="1.0"/></p>	<p><b>Fluorescence</b></p> <p>Fluorophore Name: <input type="text" value="CD8-PE"/></p> <p>Exposure (ms): <input type="text" value="600"/></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: <input type="button" value="No"/> <input type="button" value="Yes"/></p> <p>Custom Exposure Factor: <input type="text" value="1.0"/></p>	<p><b>Fluorescence</b></p> <p>Fluorophore Name: <input type="text" value="CD3-APC"/></p> <p>Exposure (ms): <input type="text" value="5000"/></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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## 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\_2SM+Total\_CD3-APC + CD8-PE + Hoechst

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

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

Imaging and Analysis

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

**Cell Type Parameters** 2SM+Total\_CD3-APC + CD8-PE + Hoechst

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, anti-human CD3 APC / CD8 PE Total Cell Kit

### Brightfield Parameters

Cell Attributes	Decustering	Trypan Blue
Cell Diameter (µm): <input type="text" value="2.0"/> to <input type="text" value="22.0"/>	<input type="button" value="No"/> <input checked="" type="button" value="Yes"/>	Dead Cell Diameter (µm): <input type="text" value="4.0"/> to <input type="text" value="50.0"/>
Roundness: <input type="text" value="0.05"/>	Edge Factor: <input type="text" value="0.7"/>	Sensitivity: <input type="text" value="1.0"/>
Contrast Enhancement: <input type="text" value="0.80"/>	Threshold Factor: <input type="text" value="1.0"/>	Uniformity: <input type="text" value="150"/>
	Background Adjustment: <input type="text" value="1.0"/>	Very Dim Dead Cells: <input checked="" type="button" value="No"/> <input type="button" value="Yes"/>
		Contrast Enhancement: <input type="text" value="0.60"/>

### Fluorescence Parameters

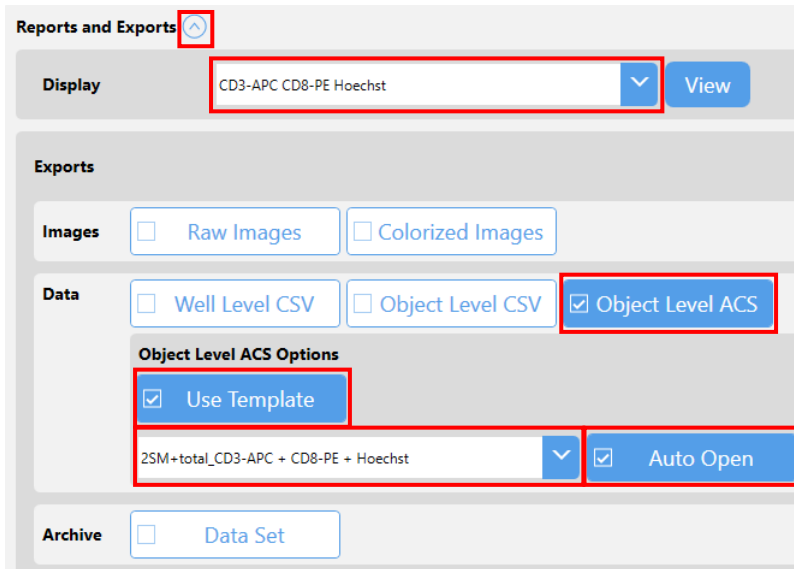
Cell Attributes	Thresholding
Cell Diameter (µm): <input type="text" value="4.0"/> to <input type="text" value="50.0"/>	<input checked="" type="button" value="Manual"/> <input type="button" value="Auto"/>
Normalize intensity for cell size: <input type="button" value="No"/> <input checked="" type="button"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



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

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 surface marker 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 Surface Marker and Total Dye Exposure Times and Filter Pairs

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Recommended imaging parameters and exposure times (with ranges) for CD3 and CD8 surface markers with Hoechst on Cellaca® PLX Low Fluorescence slides. Exposure times may require optimization due to the individuality of each patient sample or cell line.

<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	<b>600</b> (400 – 800)
531 / 605	Orange	CD8 PE	<b>600</b> (400 – 1,000)
620 / 692	Far Red	CD3 APC	<b>5,000</b> (4,000 – 8,000)





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