

PRODUCT INSERT

Instrument Compatibility:

Cellaca[®] PLX

Cellaca[®] PLX, anti-human CD3 PE Antibody

Part number:	CS1-A0002-1	CS1-A0002-2
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Test number:	25 Tests	100 Tests
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Storage: 4°C

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1. Introduction

1.1. Description

CD3 single surface marker reagent is designed for researchers interested in acquiring data on a single surface marker population, as each patient and cell line derived sample can be unique. The Cellaca® PLX provides users with fluorescent and bright field images of their CD3 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. Reagent

This antibody assesses the CD3 population on the Cellaca® PLX. The anti-human CD3 reagent is conjugated with PE. See table below for surface marker antibody details and its respective isotype control.

Cellaca® PLX Assay	Reagents	Catalog Number	Number of Tests
PLX.5_1SM__CD3-PE	PE anti-human CD3 (HIT3α)	CS1-A0002-1	25
		CS1-A0002-2	100
	PE Mouse IgG2a Isotype	CS1-A0005-1	100

1.3. Required Materials

- 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
- Antibodies from CS1-A0002
- Antibodies from CS1-A0005 for proper isotype control (recommended)
- 1X Phosphate Buffered Saline (PBS)
- Microcentrifuge tubes
- Cell culture media
- Cells or PBMC's

2. Staining Procedure for CD3 PE

Cellaca® PLX Assay	Reagents	Catalog Number	Number of Tests
PLX.5_1SM__CD3-PE	PE anti-human CD3 (HIT3α)	CS1-A0002-1	25
		CS1-A0002-2	100
	PE Mouse IgG2a Isotype	CS1-A0005-1	100

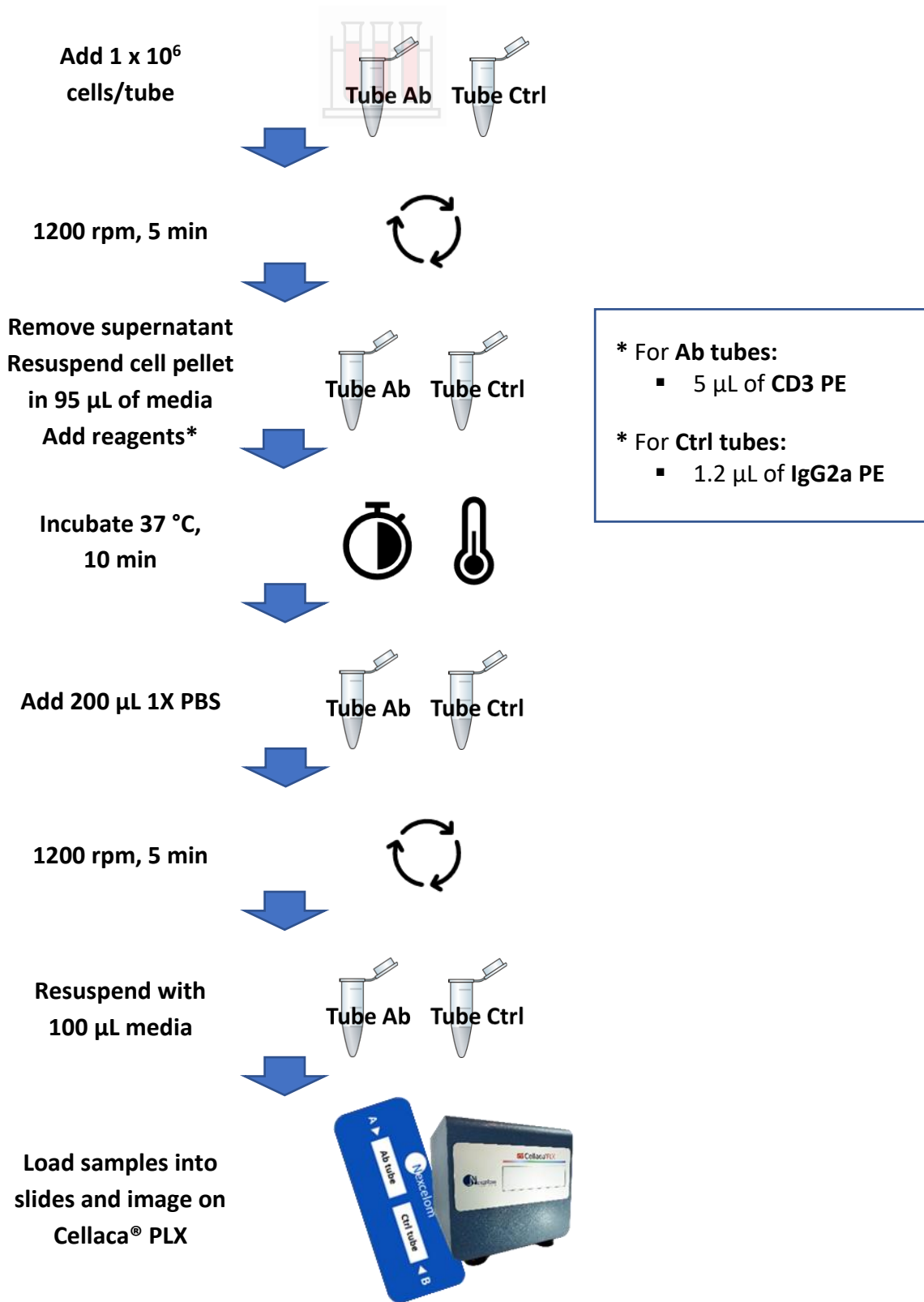
For each sample with isotype control:

1. For a single sample, prepare 2 microcentrifuge tubes with 1×10^6 PBMCs/cells each
NOTE 1: For 1×10^6 cells, take 1 mL of 1×10^6 cells/mL
NOTE 2: For multiple samples, prepare 2 tubes each
2. Label tubes, accordingly, one for staining with antibodies (**Ab**) and one for isotype control (**Ctrl**) staining for each distinct sample
3. Centrifuge cells at 1200 rpm for 5 minutes
4. Remove supernatant from all tubes avoiding cell pellets
5. Resuspend the cell pellets from all tubes in 95 μ L of cell culture media
NOTE: Staining with PBS results in dimmer signal
6. For staining cells in **Ab tubes**, add the following, and mix well:
 - 5 μ L of **CD3 PE**
7. For staining cells in **Ctrl tubes**, add the following, and mix well:
 - 1.2 μ L of **IgG2a PE**
8. Incubate all tubes in the dark for 10 minutes at 37 °C
9. To each tube, add 200 μ L of 1X PBS and mix well
10. Centrifuge cells at 1200 rpm for 5 minutes
11. Remove supernatant from each tube avoiding cell pellets
12. Resuspend each cell pellet in 100 μ L of cell culture media
NOTE: Resuspension in 1X PBS results in dimmer signal
13. Mix samples thoroughly by pipetting up and down a few times
14. Load 15 μ L 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

15. Load 15 μ L of sample from **Ctrl tube** into side B of the slide
NOTE: Repeat for any additional samples prepared
16. To image replicates from the same sample, load another slide following steps 14 and 15
17. 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
18. Proceed to section 4 for image and data acquisition



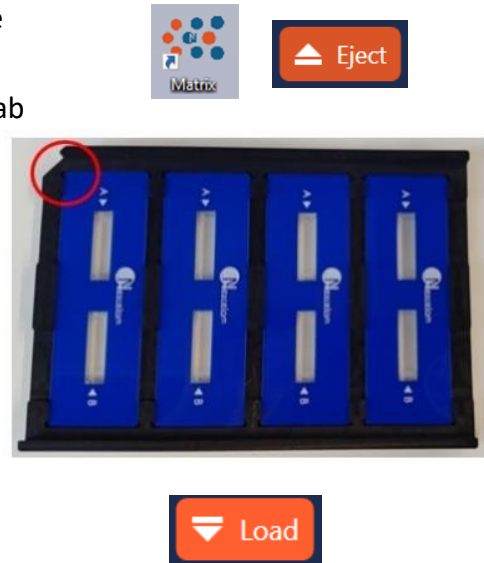
3. Expert User Quick Guide – CD3 PE



4. Cellaca® PLX Image and Data Acquisition

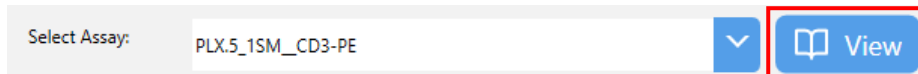
4.1. Initiate software and load samples

- 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

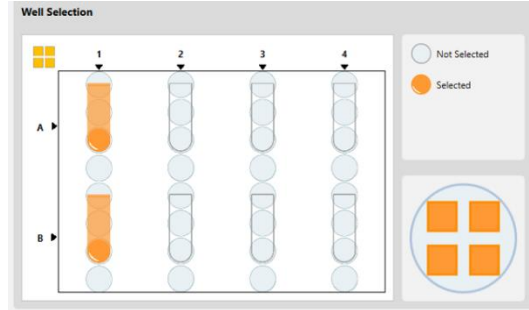
- 4.3.1. In **Well Details**:
 - 4.3.1.1. Select "4 Slides (CHM2-ACR)" as the **Plate Type**



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)

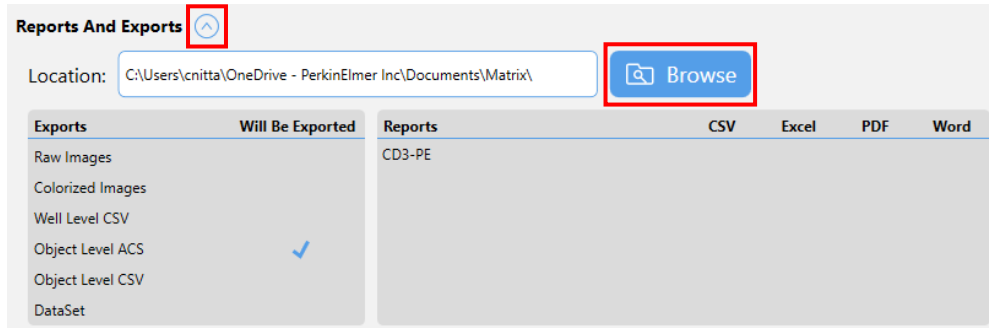


4.4. Reports and Exports

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

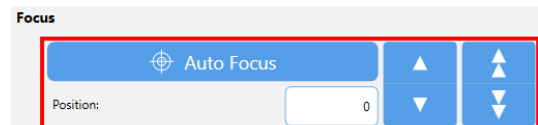


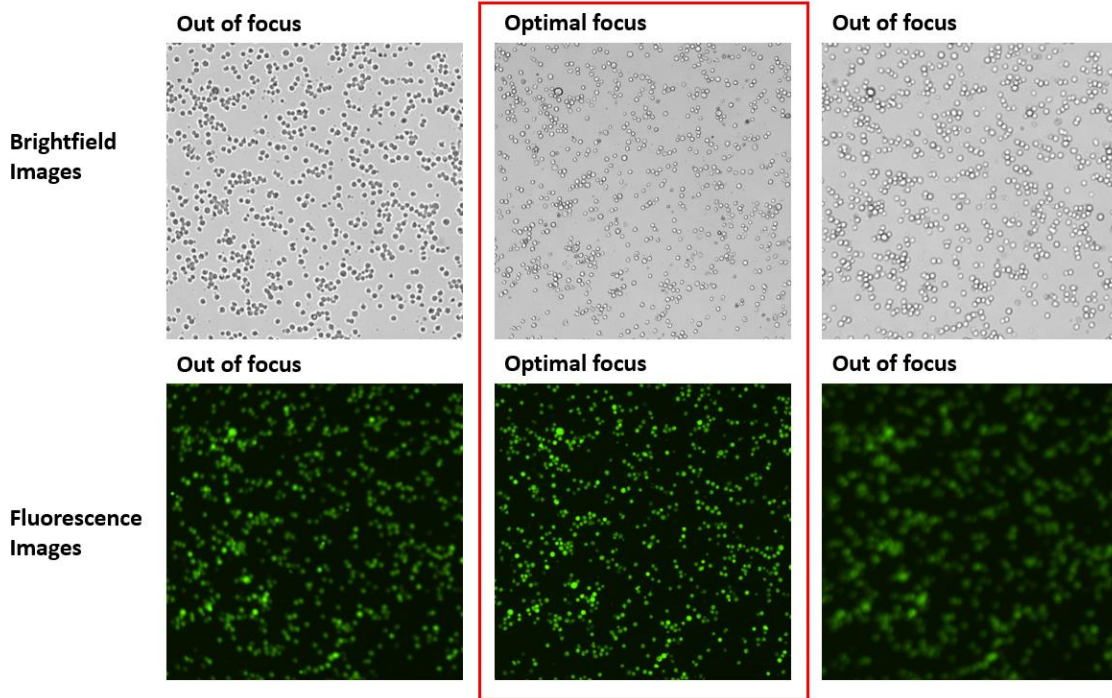
4.5. Preview Samples

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

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 fluorescence

4.5.3.1. Adjust exposure times as needed

NOTE: See Recommended Surface Marker Exposure Time and Filter Pair in the Appendix



4.5.4. 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: object_A1.acs and object_B1.acs as CD3-PE and IgG2a-PE Isotype, 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 (CD3-PE)	object_A2.acs
	2 (IgG2a-PE isotype)	object_B2.acs

5. Additional Resources

5.1. Storage / Safety

Store product at 4 °C, protected from light. Please consult the Safety Data Sheet for more safety information, found on www.revivity.com/cellcountingreagents.

5.2. 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.

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

When ordering with a Purchase Order:

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

For online orders, please visit:

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

For support, e-mail Cellc-support@revivity.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

Select Assay:

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, anti-human CD3 PE Antibody

Imaging and Analysis

Imaging Mode

BR BR/FL Number of Channels:

Two-Channel Imaging: Single Fluorescence And Brightfield Analysis

Analysis Mode

Cell Count Viability Expression

Analyze A Single Fluorophore (GFP, RFP, etc.)

Mask: BR FL

Uses the Brightfield image to aid in the finding of FL positive Cells

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 the Cellaca® PLX, anti-human CD3 PE Antibody

Channel 1

Imaging Parameters

Brightfield
Use Custom Exposure:
Custom Exposure Factor:

Fluorescence
Fluorophore Name:
Exposure (ms):

Filters
Excitation: 365 470 531 620
Emission: 452 534 605 655 692

Cell Type Parameters 1SM_CD3-PE

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_1SM_CD3-PE

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**

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

Cell Type Parameters 1SM_CD3-PE

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 PE Antibody

Brightfield Parameters

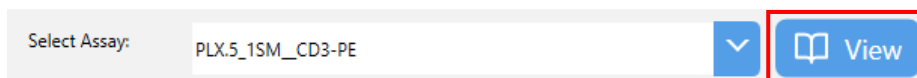
Cell Attributes	Decustering	Trypan Blue
Cell Diameter (µm): <input type="text" value="2.0"/> to <input type="text" value="22.0"/>	<input type="button" value="No"/> <input checked="" type="button" value="Yes"/>	Dead Cell Diameter (µm): <input type="text" value="4.0"/> to <input type="text" value="50.0"/>
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

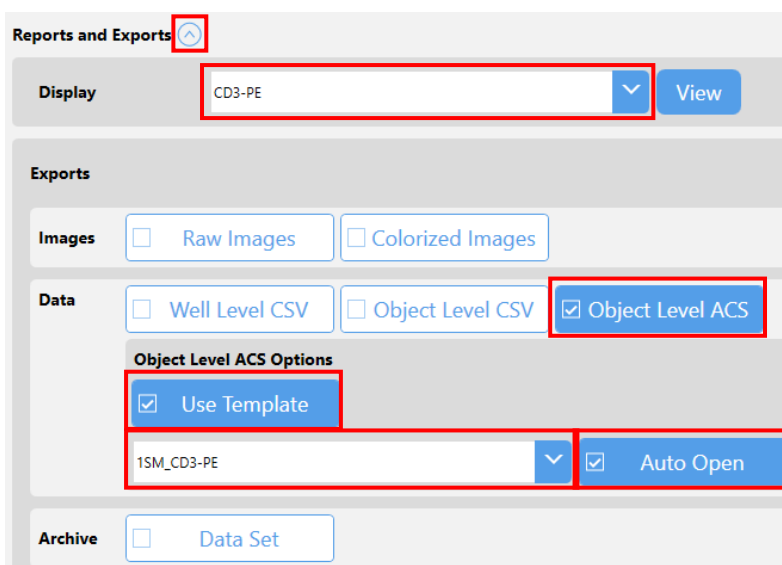
Cell Attributes	Thresholding
Cell Diameter (µm): <input type="text" value="4.0"/> to <input type="text" value="50.0"/>	<input checked="" type="button" value="Manual"/> <input type="button" value="Auto"/>
Normalize intensity for cell size: <input type="button" value="No"/> <input checked="" type="button" 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 checked="" type="button" value="No"/> <input 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 Exposure Time and Filter Pair

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

Cellaca® PLX Excitation / Emission	Illumination	Reagent	Assay Default Exposure Time (ms) (Recommended range)
531 / 605	Orange	CD3 PE	1,000 (800 – 1,500)



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