Antibodies For Cellaca® PLX

Cellaca® PLX, anti-human CD3 APC Antibody
CS1-A0003-1 (25 Tests)
CS1-A0003-2 (100 Tests)

Cellaca® PLX, APC Mouse IgG2a, κ Isotype Ctrl Antibody
CS1-A0006-1 (100 Tests)
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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 APC. See table below for surface marker antibody details and its respective isotype control.

<table>
<thead>
<tr>
<th>Cellaca® PLX Assay</th>
<th>Reagents</th>
<th>Catalog Number</th>
<th>Number of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLX.5_1SM__CD3-APC</td>
<td>APC anti-human CD3 (HIT3α)</td>
<td>CS1-A0003-1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CS1-A0003-2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>APC Mouse IgG2a Isotype</td>
<td>CS1-A0006-1</td>
<td>100</td>
</tr>
</tbody>
</table>

1.3. Required Materials
1.3.1. Cellaca® PLX instrument
1.3.2. Nexcelom-provided Laptop with Matrix 5.0 Software or above (pre-installed)
1.3.3. FCS Express software (pre-installed on Nexcelom-provided laptop)
1.3.4. Cellaca® PLX Low Fluorescence Slides (Cat. # CHM2-ACR)
1.3.5. Cellaca® PLX slide holder
1.3.6. Antibodies from CS1-A0003
1.3.7. Antibodies from CS1-A0006 for proper isotype control (recommended)
1.3.8. 1X Phosphate Buffered Saline
1.3.9. Microcentrifuge tubes
1.3.10. Cell culture media
1.3.11. Cells or PBMC’s
2. Staining Procedure for CD3 APC

<table>
<thead>
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<td></td>
<td>APC Mouse IgG2a Isotype</td>
<td>CS1-A0006-1</td>
<td>100</td>
</tr>
</tbody>
</table>

For each sample with isotype control:

1. 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

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 µL 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 µL of CD3 APC

7. For staining cells in Ctrl tubes, add the following, and mix well:
   
   - 1.2 µL of IgG2a APC

8. Incubate all tubes in the dark for 30 minutes at 4 °C

9. To each tube, add 200 µL 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 µL 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 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

15. Load 15 µL 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.

Add 1 x 10^6 cells/tube

1200 rpm, 5 min

Remove supernatant
Resuspend cell pellet in 95 µL of media
Add reagents *

Incubate 4°C, 30 min

Add 200 µL 1X PBS

1200 rpm, 5 min

Resuspend with 100 µL media

Load samples into slides and image on Cellaca® PLX

* For Ab tubes:
  ▪ 5 µL of CD3 APC

* For Ctrl tubes:
  ▪ 1.2 µL of IgG2a APC
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 time 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-APC and IgG2a-APC 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.
5. Additional Resources

5.1. Storage and Handling
Store product at 4 °C, protected from light. Please consult the Safety Data Sheet for more safety information, found on www.nexcelom.com/Products.

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:
   Fax a copy of the order to 978-327-5341
   Email a copy of the order to Cellc-sales@perkinelmer.com
   Email support at Cellc-support@perkinelmer.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 Antibody.
NOTE: Below are the default Imaging Parameters for the Cellaca® PLX, anti-human CD3 APC Antibody

6.2. Cell Type Parameters

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

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

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 Antibody

Brightfield Parameters

<table>
<thead>
<tr>
<th>Cell Attributes</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Diameter (um)</td>
<td>2.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Roundness</td>
<td>0.05</td>
<td></td>
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<tr>
<td>Contrast Enhancement</td>
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Declustering

<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
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<tbody>
<tr>
<td>Edge Factor</td>
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<tr>
<td>Threshold Factor</td>
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<tr>
<td>Background Adjustment</td>
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Trypan Blue

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Dead Cell Diameter (um)</td>
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<tr>
<td>Sensitivity</td>
<td>1.0</td>
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<tr>
<td>Uniformity</td>
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Fluorescence Parameters

<table>
<thead>
<tr>
<th>Cell Attributes</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Diameter (um)</td>
<td>4.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Normalize intensity for cell size</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-Uniform Cells</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Roundness</td>
<td>0.10</td>
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</tr>
<tr>
<td>Do Not Count Nuclei</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Advanced BR/P Mode</td>
<td>No</td>
<td>Yes</td>
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</table>

Thresholding

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Image Range to Count</td>
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</tr>
<tr>
<td>Threshold Factor</td>
<td>1.0</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Cellaca® PLX Excitation / Emission</th>
<th>Illumination</th>
<th>Reagent</th>
<th>Assay Default Exposure Time (ms) (Recommended range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>620 / 692</td>
<td>Far Red</td>
<td>CD3 APC</td>
<td>5,000 (4,000 – 8,000)</td>
</tr>
</tbody>
</table>