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# **Simplify Immunophenotyping Using Kits and Templates on the BD Accuri™ C6**

**Presented by:**

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# Outline

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- Background: Immunophenotyping
- Introduction to the BD Accuri™ C6 Flow Cytometer
- BD Kits and Templates
- BD Templates on the Web
- How to Create Your Own Templates
- Coming Soon....



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# Outline

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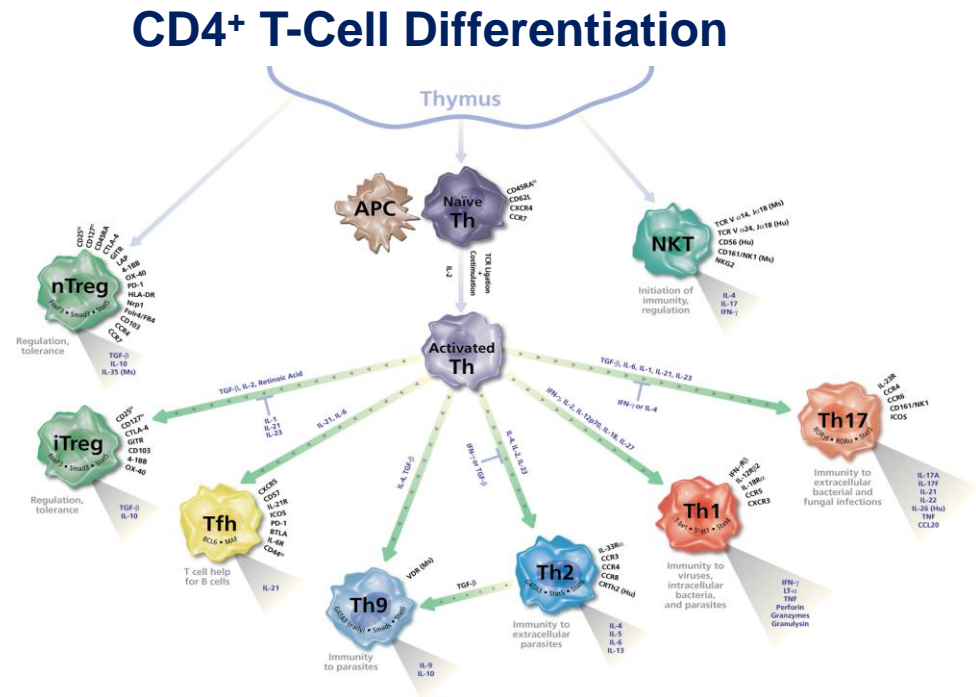
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# T-Cell Immunophenotyping

- **T Cells:** Specialized subset of lymphocytes that mediate antigen-specific immune responses
- **Main Subsets:**
  - **CD4<sup>+</sup> T Helper Cells** regulate the adaptive immune response; lack intrinsic cytotoxic or phagocytic ability.
  - **CD8<sup>+</sup> Cytotoxic T Cells** can directly kill infected cells via perforin and granzymes.
  - Each of these subsets can be further broken down to more specialized cell types.



# CD4<sup>+</sup> T-Cell Subsets

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- **Th1**: Activate infected macrophages and help B cells; immunity to viruses, intracellular bacteria, and parasites
- **Th2**: Help B cells and switch antibody production; immunity to extracellular parasites
- **Th17**: Inflammation and autoimmunity; immunity to extracellular bacteria and fungi
- **Regulatory T cells (Tregs)**: Regulate the immune response (inhibition or downregulation)



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# CD4 T-Cell Phenotypes/Subsetting

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- Different CD4 T-cell phenotypes can be defined by cell surface markers, expression of transcription factors, and cytokine production.
- Many CD4 subsets cannot be detected using cell surface markers alone.
  - For example, identification of Th17 cells requires the detection of the IL-17 cytokine or ROR $\gamma$ T transcription factor.
  - Detection of intracellular markers by flow cytometry requires special consideration.
  - Flow cytometry allows the study of multiple subsets within the same sample simultaneously.



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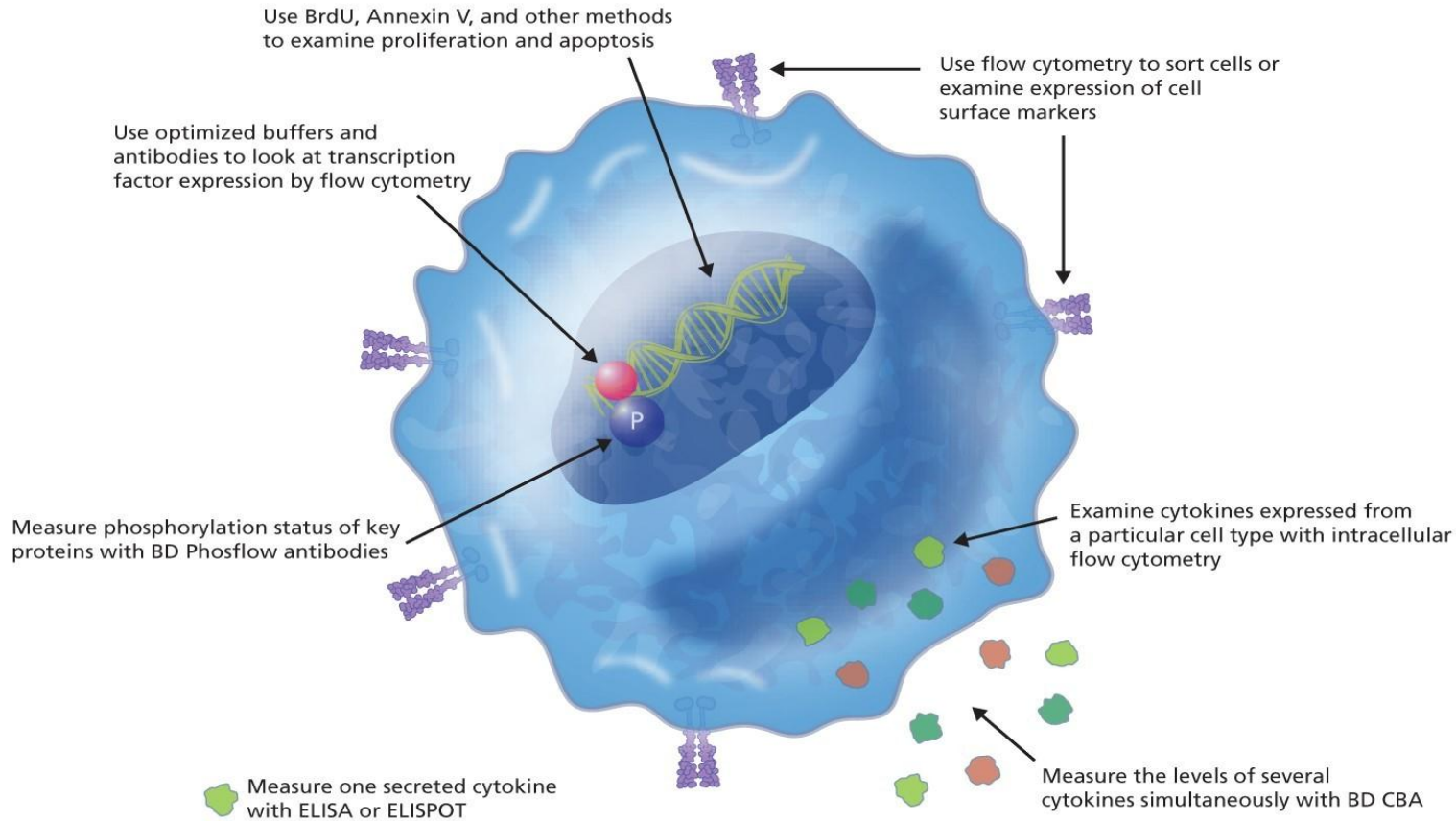
# Key T-Cell Surface Markers and Cytokines

| Marker        | Significance                                |
|---------------|---|
| CD3           | T-cell lineage marker, all T cells are CD3+ |
| CD4           | Marker for T helper cells                   |
| CD8           | Marker for cytotoxic T cells                |
| IFN- $\gamma$ | Th1 signature cytokine                      |
| IL-4          | Th2 signature cytokine                      |
| IL-17A        | Th17 signature cytokine                     |
| CD45RA        | Marker for naïve T cells                    |
| CD45RO        | Marker for memory T cells                   |
| CD62L         | Marker for memory cell activation           |
| CD197         | Marker for central/effector memory cells    |



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# Flow Cytometry Allows the Detection of Multiple Parameters





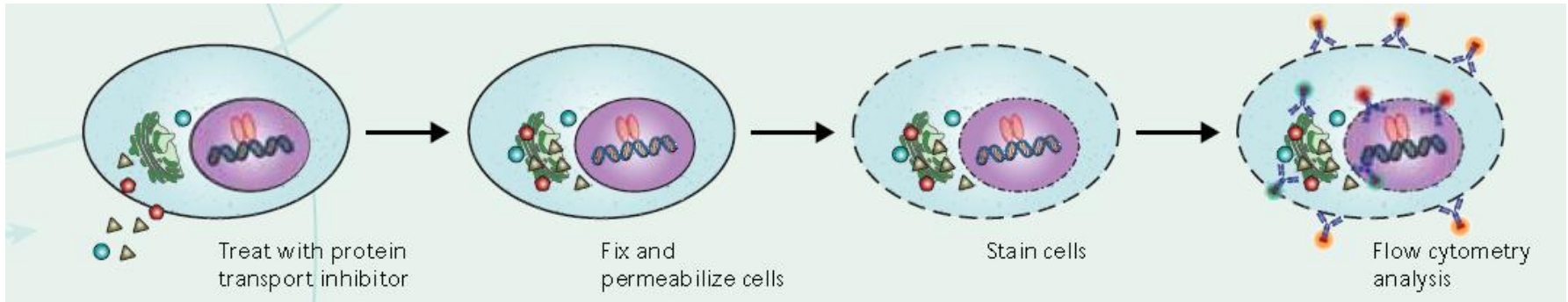
# Methods to Detect Cytokines

| Capability  | BD CBA  | ELISA  | Intracellular Flow  |
|---|---|--|---|
| Allows the detection of multiple cytokines in the same experiment                 |  |  |  |
| Can obtain the phenotype of specific cells expressing the cytokine(s) of interest |   |  |  |
| Can measure the quantity of the cytokine secreted                                 |  |  |   |



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# Detection of Cytokines by Intracellular Flow



- Cytokines are secreted proteins that need to be trapped inside the cell, fixed, and permeabilized for detection by intracellular flow.
- Cytokines can be trapped inside the cell using a protein transport inhibitor such as BD GolgiStop™ (Monensin) or BD GolgiPlug™ (Brefeldin A).
- BD kits contain the appropriate protein transport inhibitor, buffers, cocktails, and detailed protocols to simplify experiments and reduce variability across experiments and laboratories.



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# Introduction

## The BD Accuri™ C6 Flow Cytometer System



**An Affordable, Full-Featured, Easy-to-Use Flow Cytometer**

**Two lasers and six detectors**



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# Intuitive Software

Sample Grid

Cytometer Status

Fluidics Controls

Run Criteria

Real-Time Updates

The screenshot displays the BD CSampler software interface with the following components:

- Menu Bar:** File, Edit, Display, Instrument, About
- Navigation Tabs:** Manual Collect, Auto Collect, Analyze, Statistics, Batch Analysis
- Control Panel (Left):**
  - Plate Type: 24 tube rack
  - Eject Plate button
  - Plate Name: Click here to name plate
  - Panel 3 grid (A1-A12, H1-H12) with color-coded wells
  - Cyometer status: Cs is connected and ready.
  - Run Settings: Run Unlimited, Run with Limits (10000 events in Lymphs)
  - Fluidics: Flow Rate 60 µL/min, Core Size 22 µm
  - Threshold: Set Threshold, 250,000 on FSC-H
  - Buttons: Backflush, Unclog, Wash, Agitate
  - ADD to C01 button
  - Last Run Summary: 0 Events, 170.136 Time, 4:31.7
- Plot Area (Center):**
  - Plot 1: C01 Panel 3 (GATE) [No Gating] - Scatter plot of SSC-A vs FSC-A with a red gate for Lymphs (38.0%).
  - Plot 2: C01 Panel 3 (GATE) (Lymphs in all) - Scatter plot of FL2-A vs FL1-A with gates P2 (9.1%) and P3 (80.2%).
  - Plot 3: C01 Panel 3 (GATE) (Lymphs in all) - Histogram of FL4-A.
  - Plot 4: C01 Panel 3 (GATE) (Lymphs in all) - Scatter plot of FL1-A vs FL2-A.
  - Plot 5: Placeholder for a new plot.
  - Plot 6: Placeholder for a new plot.
  - Plot 7: C01 Panel 3 (GATE) (Lymphs in all) - Placeholder for a new plot.
- Statistics Tables (Bottom):**

| Plot 2: C01 Panel 3      | Count  | Volume (µL) | % of This Plot | % of All | Mean FL1-A | Mean FL2-A | CV FL1-A | CV FL2-A | Median FL1-A | Median FL2-A |
|--------------------------|--------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| Gated on (Lymphs in all) |        |             |                |          |            |            |          |          |              |              |
| This Plot                | 64,614 | 297         | 100.00%        | 37.98%   | 33,627.31  | 4,263.52   | 77.45%   | 137.12%  |              |              |
| P2                       | 5,896  | 297         | 9.12%          | 3.47%    | 2,400.14   | 15,828.54  | 28.98%   | 35.55%   |              |              |
| P3                       | 51,839 | 297         | 80.23%         | 30.47%   | 41,321.67  | 3,420.89   | 32.62%   | 31.27%   |              |              |

| Plot 6: C01 Panel 3      | Count  | Volume (µL) | % of This Plot | % of All | Mean FL4-A | Median FL4-A |
|--------------------------|--------|-------------|----------------|----------|------------|--------------|
| Gated on (Lymphs in all) |        |             |                |          |            |              |
| This Plot                | 64,614 | 297         | 100.00%        | 37.98%   | 17,542.71  | 202.67%      |

| Plot 7: C01 Panel 3      | Count  | Volume (µL) | % of This Plot | % of All | Mean FL2-A | Mean FL1-A | CV FL2-A | CV FL1-A | Median FL2-A | Median FL1-A |
|--------------------------|--------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| Gated on (Lymphs in all) |        |             |                |          |            |            |          |          |              |              |
| This Plot                | 64,614 | 297         | 100.00%        | 37.98%   | 4,263.52   | 33,627.31  | 137.12%  | 77.45%   |              |              |

Histograms

Dot Plots

Density Plots

Analysis and Gating Tools

Plot Statistics



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# BD Kits and Templates

The BD Accuri™ C6 Personal Flow Cytometer  
Conduct powerful assays in just a few easy steps.

The image illustrates the workflow for using the BD Accuri C6 Personal Flow Cytometer. It is divided into three numbered steps: 1. The cytometer unit, a white and red device with a sample inlet and various tubes. 2. Reagents and kits, including a blue BD box, a clear plastic bottle, and two small vials. 3. Data analysis, shown as two tablet screens displaying flow cytometry plots. The first plot shows a single peak with 'Cells 100.0%' and axes labeled 'SSC-A' and 'FSC-A'. The second plot shows a gated population with 'Cells 91.5%' and axes labeled 'SSC-A' and 'FSC-A'. Red arrows indicate the flow from the reagents to the cytometer and then to the data analysis screens.

Arguably, as easy as cell analysis is going to get.



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# BD Immunology Kits

| Application          | Kit                                | Cat. No.      |
|----------------------|------------------------------------|---------------|
| Naïve/Memory T Cell  | CD45RA/CD45RO/<br>CD3/CD4          | <u>340571</u> |
|                      | CD45RA/CD62L/CD3/CD4               | <u>340977</u> |
|                      | Human Naïve/Memory<br>T Cell Panel | <u>561438</u> |
| Intracellular T Cell | Th1/Th2/Th17<br>Phenotyping Kit    | <u>560751</u> |
|                      | IFN- $\gamma$ /CD69/CD8/CD3 Kit    | <u>346048</u> |
|                      | IFN- $\gamma$ /IL4 Kit             | <u>340456</u> |



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# What is a Template?

The screenshot displays the BD Accuri C6 software interface. On the left, there is a 'Collect' panel with a 12x8 grid of sample wells (A1-H12) and a 'RUN' button. Below the grid are 'Run Settings' (Run Unlimited/with Limits, events, thresholds) and 'Fluidics' (Flow Rate, Core Size) sections. The main area shows 'Statistics' and 'Batch Analysis' tabs. Three plots are visible: Plot 1 (SSC-A vs FSC-A) with a 'Lymphocytes' gate, Plot 2 (SSC-A vs FL3 CD4 PerCP Cy5.5-A) with a 'CD4+' gate, and Plot 3 (FL4 CD197 AF 647-A vs FL1 CD45RA FITC-A) with 'Central Memory', 'Naive', 'Effector Memory', and 'Q1-LR' populations. Below the plots are three prompts: 'Select plot type to make a new plot.' with icons for histogram, scatter, and contour plots. At the bottom, there are three data tables for the plots.

| Plot 1: C09 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC-A | Median SSC-A |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| All         | 0     | 0           | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |              |              |
| Lymphocytes | 0     | 0           | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |              |              |

| Plot 4: C09 | Count | Volume (µL) | % of This Plot | % of All | Mean FL3 CD4 PerCP Cy5.5-A | Mean SSC-A | CV FL3 CD4 PerCP Cy5.5-A | CV SSC-A |
|-------------|-------|-------------|----------------|----------|----------------------------|------------|--------------------------|----------|
| All         | 0     | 0           | 100.00%        | 100.00%  | 0.00                       | 0.00       | 0.00%                    | 0.00%    |
| CD4+        | 0     | 0           | 100.00%        | 100.00%  | 0.00                       | 0.00       | 0.00%                    | 0.00%    |

| Plot 3: C09    | Count | Volume (µL) | % of This Plot | % of All | Mean FL1 CD45RA FITC-A | Mean FL4 CD197 AF 647-A | CV FL1 CD45RA FITC-A |
|----------------|-------|-------------|----------------|----------|------------------------|-------------------------|----------------------|
| This Plot      | 0     | 0           | 100.00%        | 100.00%  | 0.00                   | 0.00                    | 0.00%                |
| Central Memory | 0     | 0           | 100.00%        | 100.00%  | 0.00                   | 0.00                    | 0.00%                |

A BD Accuri template is a predefined workspace that includes gates, labels, run criteria, and compensation settings for a specific assay.



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# Predefined Gating and Run Criteria for Ease of Use

**BD Accuri™ C6**

Collect
Analyze
Statistics
Batch Analysis

**C09**

|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  | 11  | 12  |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

Plot 1: C09 GATE [No Gating]

Plot 4: C09 GATE [No Gating]

Plot 3: C09 GATE [CD4+ in all]

Select plot type  
to make a new plot.

Select plot type  
to make a new plot.

Select plot type  
to make a new plot.

**Run Settings**

Run Unlimited

Run with Limits

Events: 0

Min: 30 Sec

Do not collect events outside: Lymphocytes

Backflush Unclog

RUN

**Fluidics**

Flow Rate: 30 µL/min

Core Size: 10 µm

Flow Rate: 14 µL/min

Core Size: 10 µm

Set Core Size

**Threshold**

Set Threshold: 80,000 on FSC-A

**Last Run**

0 Events

0.00 Time

0 Microliters

0 Events / Sec

0 Events / µL

**Cumulative**

0 Events

0.00 Time

0 Microliters

0 Events / Sec

0 Events / µL

**Delete Events**

All

Outside: Lymphocytes

Data Capacity Used: 0% of 99,000,000 Events

| Plot 1: C09 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC-A | Median SSC-A |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| All         | 0     | 0           | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |              |              |
| Lymphocytes | 0     | 0           | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |              |              |

| Plot 4: C09 | Count | Volume (µL) | % of This Plot | % of All | Mean FL3 CD4 PerCP Cy5.5-A | Mean SSC-A | CV FL3 CD4 PerCP Cy5.5-A | CV SSC-A |
|-------------|-------|-------------|----------------|----------|----------------------------|------------|--------------------------|----------|
| All         | 0     | 0           | 100.00%        | 100.00%  |                            | 0.00       | 0.00                     | 0.00%    |
| CD4+        | 0     | 0           | 100.00%        | 100.00%  |                            | 0.00       | 0.00                     | 0.00%    |

| Plot 3: C09            | Count | Volume (µL) | % of This Plot | % of All | Mean FL1 CD45RA FITC-A | Mean FL4 CD197 AF 647-A | CV FL1 CD45RA FITC-A |
|------------------------|-------|-------------|----------------|----------|------------------------|-------------------------|----------------------|
| Gated on (CD4+ in all) |       |             |                |          |                        |                         |                      |
| This Plot              | 0     | 0           | 100.00%        | 100.00%  | 0.00                   | 0.00                    | 0.00%                |
| Central Memory         | 0     | 0           | 100.00%        | 100.00%  | 0.00                   | 0.00                    | 0.00%                |



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# Compensation

BD Accuri™ C6

Collect Analyze Statistics Batch Analysis

H12

|   |    |    |    |    |    |    |    |    |    |     |     |     |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11  | 12  |     |
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

Run Settings

Run Unlimited  Run with Limits

Fluidics

Slow  Medium  Fast

Flow Rate 86 µL/min  
Core Size 22 µm

Custom

Flow Rate 14 µL/min  
Set Core Size  
Core Size 10 µm

Threshold

80,000 on FSC-H  
None

Set Threshold

Backflush Unclog

RUN

Set Color Compensation

Last Run Cumulative

Events 0  
Time 0:00:0  
Microliters 0  
Events / Sec 0  
Events / µL 0

Delete Events  show warning

All  Outside  Lymphs

Data Capacity Used  
0% of 96,000,000 Events

Plot 1: A01 GATE [No Gating]

Plot 2: A01 GATE [Lymphs in all]

Plot 3: A01 GATE [CD4+ in (Lymphs in all...)]

Compensation Settings for A01

Correct FL1 by subtracting a percentage of:

FL2: 4.68 % FL3: 0.00 % FL4: 0.00 %

Correct FL2 by subtracting a percentage of:

FL1: 0.10 % FL3: 0.22 % FL4: 0.00 %

Correct FL3 by subtracting a percentage of:

FL1: 0.00 % FL2: 21.00 % FL4: 0.00 %

Correct FL4 by subtracting a percentage of:

FL1: 0.00 % FL2: 0.00 % FL3: 9.20 %

Apply to:

Reset all to 0.00%  A01  All samples

Preview Apply & Close Cancel & Close

Select plot type to make a new plot.

|        | CV FSC-A | CV SSC-A | Median FSC-A | Median SSC-A |
|--------|----------|----------|--------------|--------------|
| All    | 0        | 0        | 100.00%      | 100.00%      |
| Lymphs | 0        | 0        | 100.00%      | 100.00%      |

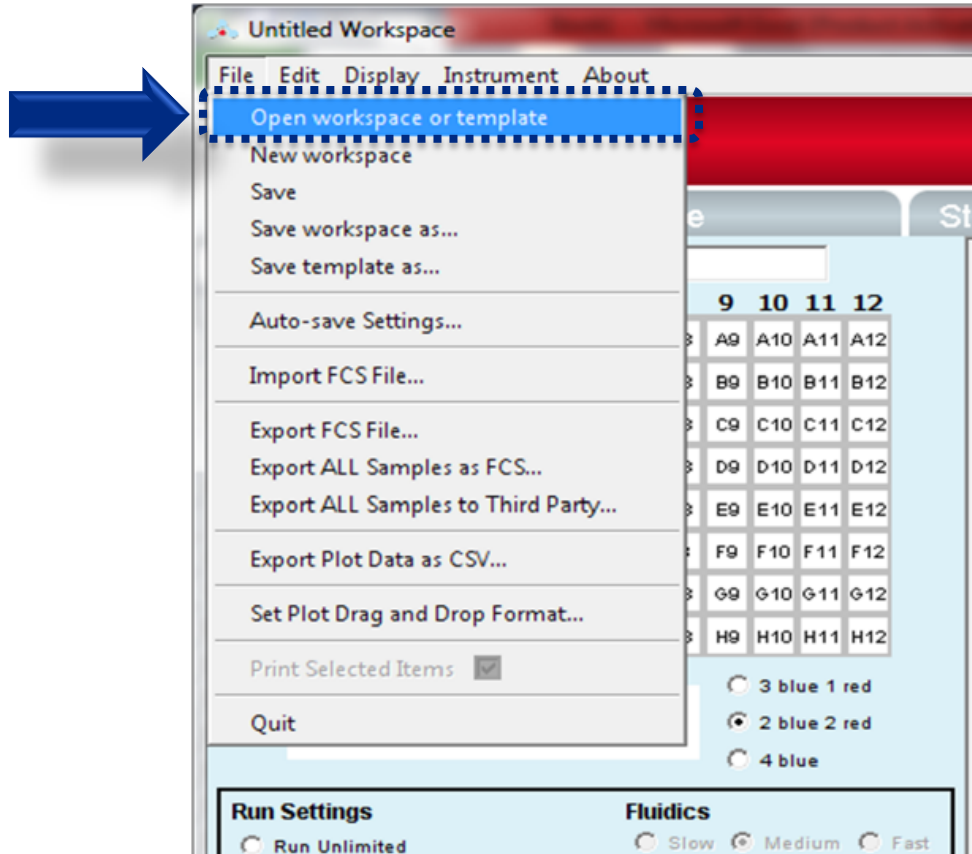
|                                      | Count | Volume (µL) | % of This Plot | % of All | Mean FL3 CD4 PerCP-Cy5.5-A | CV FL3 CD4 PerCP-Cy5.5-A | Median FL3 CD4 |
|--------------------------------------|-------|-------------|----------------|----------|----------------------------|--------------------------|----------------|
| Plot 2: A01 Gated on (Lymphs in all) |       |             |                |          |                            |                          |                |
| This Plot                            | 0     | 0           | 100.00%        | 100.00%  | 0.00                       | 0.00%                    | 0.00%          |
| CD4+ (1,983.0 / 15,40...)            | 0     | 0           | 100.00%        | 100.00%  | 0.00                       | 0.00%                    | 0.00%          |

|  | Count | Volume (µL) | % of This Plot | % of All | Mean FL4 IL-4 APC-A | Mean FL2 IL-17A PE-A | CV FL4 IL-4 APC-A |
|--|-------|-------------|----------------|----------|---------------------|----------------------|-------------------|
| Plot 3: A01 Gated on (CD4+ in (Lymphs in all)) |       |             |                |          |                     |                      |                   |
| This Plot                                      | 0     | 0           | 100.00%        | 100.00%  | 0.00                | 0.00                 | 0.00%             |
| Q1-UL  | 0     | 0           | 100.00%        | 100.00%  | 0.00                | 0.00                 | 0.00%             |



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# How to Open a Template



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# How to Open a Template

The screenshot displays the BD Accuri C6 software interface. The main window is titled 'Untitled Workspace' and features a menu bar (File, Edit, Display, Instrument, About) and a red header with the product name. Below the header are tabs for 'Collect', 'Analyze', 'Statistics', and 'Batch Analysis'. The 'Collect' tab is active, showing a grid of sample wells (A1-A12, B1-B12, etc.) and a 'Plot 1: A01 (GATE) [No Gating]' window. A blue arrow points from the 'Open' dialog box to the 'Run Settings' panel. The 'Open' dialog box is open, showing a file list under 'Look In: Templates'. The file list includes: Active caspase-3 PE\_550914 template\_24 tube rack, Annexin V FITC\_556570 template\_24 tube rack, Annexin V PE\_559763 template, Bacterial Viability\_BD Cell Viab kit, BD FastImmune\_340456 template\_24 tube rack, and BD FastImmune\_346048 template. The 'File Name' field contains 'Active caspase-3 PE\_550914 template\_24 tube rack.c6t' and the 'Files of type' is set to 'Workspace and Template (\*.c6, \*.c6t, \*.c6f, \*.c6i)'. The 'Open' button is highlighted.

Run Settings

- Run Unlimited
- Run with Limits
- 10000 events
- In Ungated Sample
- 0 Min 0 Sec
- 0  $\mu$ L
- Do not collect events outside

Fluidics

- Flow Rate:  $\mu$ L/min
- Gate Size:  $\mu$ m
- Custom
- Flow Rate:  $\mu$ L/min
- Gate Size:  $\mu$ m

Threshold

- Set Threshold
- 80,000 on FSC-H
- None

| Plot 1: A01 | Count | Volume ( $\mu$ L) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A |
|-------------|-------|-------------------|----------------|----------|------------|------------|----------|----------|
| All         | 0     | 0                 | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |



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# How to Use a Template: Example Using Human Naïve/Memory T Cell Panel (Cat. No. 561438)

**BD Accuri™ C6**

Collect Analyze Statistics Batch Analysis

C09

|   |    |    |    |    |    |    |    |    |    |     |     |     |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

**Run Settings**

Run Unlimited

Run with Limits

0 events

in Ungated Sample

0 Min 30 Sec

0 µL

Do not collect events outside

Lymphocytes

Backflush Unclog

**Fluidics**

Slow  Medium  Fast

Flow Rate 35 µL/min

Core Size 16 µm

Custom

Flow Rate 14 µL/min

Set Core Size

Core Size 10 µm

**Threshold**

Set Threshold

80,000 on FSC-H

None

**RUN**

Set Color Compensation

**Last Run**

0 Events

0:00.0 Time

0 Microliters

0 Events / Sec

0 Events / µL

**Cumulative**

0 Events

0:00.0 Time

0 Microliters

0 Events / Sec

0 Events / µL

**Delete Events**  show warning

All

Outside Lymphocytes

Data Capacity Used

0% of 98,000,000 Events

Plot 1: C09 (GATE) [No Gating]

SSC-A

FSC-A

Lymphocytes 100.0%

Plot 4: C09 (GATE) [No Gating]

SSC-A

FL3 CD4 PerCP Cy5.5-A

CD4+ 100.0%

Plot 3: C09 (GATE) (CD4+ in all)

FL4 CD197 AF 647-A

FL1 CD45RA FITC-A

Central Memory 100.0%

Naive 100.0%

Effector Memory

O1-LR 100.0%

Select plot type to make a new plot.

Select plot type to make a new plot.

Select plot type to make a new plot.

| Plot 1: C09 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC-A | Median SSC-A |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| All         | 0     | 0           | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |              |              |
| Lymphocytes | 0     | 0           | 100.00%        | 100.00%  | 0.00       | 0.00       | 0.00%    | 0.00%    |              |              |

| Plot 4: C09 | Count | Volume (µL) | % of This Plot | % of All | Mean FL3 CD4 PerCP Cy5.5-A | Mean SSC-A | CV FL3 CD4 PerCP Cy5.5-A | CV SSC-A |
|-------------|-------|-------------|----------------|----------|----------------------------|------------|--------------------------|----------|
| All         | 0     | 0           | 100.00%        | 100.00%  | 0.00                       | 0.00       | 0.00%                    | 0.00%    |
| CD4+        | 0     | 0           | 100.00%        | 100.00%  | 0.00                       | 0.00       | 0.00%                    | 0.00%    |

| Plot 3: C09            | Count | Volume (µL) | % of This Plot | % of All | Mean FL1 CD45RA FITC-A | Mean FL4 CD197 AF 647-A | CV FL1 CD45RA FITC-A | CV FL4 CD197 AF 647-A |
|------------------------|-------|-------------|----------------|----------|------------------------|-------------------------|----------------------|-----------------------|
| Gated on (CD4+ in all) |       |             |                |          |                        |                         |                      |                       |
| This Plot              | 0     | 0           | 100.00%        | 100.00%  | 0.00                   | 0.00                    | 0.00%                | 0.00%                 |
| Central Memory         | 0     | 0           | 100.00%        | 100.00%  | 0.00                   | 0.00                    | 0.00%                | 0.00%                 |

Panel:  
**CD4 PerCP-Cy™ 5.5**  
**CD45RA FITC**  
**CD197 Alexa**  
**Fluor® 647**



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# How to Use a Template: Verify Settings, Collect, Adjust as Necessary

BD Accuri™ C6

Collect Analyze Statistics Batch Analysis

B01 Naive Memory panel 561438

|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  | 11  | 12  |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

**Run Settings**

Run Unlimited  
 Run with Limits

0 events in Ungated Sample  
 0 Min 30 Sec  
 Do not collect events outside Lymphocytes

**Fluidics**

Slow  Medium  Fast  
 Flow Rate 06 µL/min  
 Core Size 22 µm  
 Custom  
 Flow Rate 14 µL/min  
 Core Size 10 µm

**Threshold**

Set Threshold  
 80,000 on FSC-H  
 None

**ADD to B01**

Set Color Compensation

Last Run Cumulative  
 0 Events 90,411  
 0:00.0 Time 2:01.5  
 0 Microliters 133  
 0 Events / Sec 743  
 0 Events / µL 680

Delete Events  show warning  
 All  
 Outside Lymphocytes  
 Data Capacity Used <1% of 96,000,000 Events

Plot 1: B01 Naive Memory panel 561438  
GATE [No Gating]

Plot 4: B01 Naive Memory panel 561438  
GATE [No Gating]

Plot 3: B01 Naive Memory panel 561438  
GATE [CD4+ in all]

Select plot type to make a new plot.

| Plot 4: B01 Naive Memory panel 561438 | Count  | Volume (µL) | % of This Plot | % of All | Mean FL3 CD4 PerCP Cy5.5-A | Mean SSC-A | CV FL3 CD4 P |
|---------------------------------------|--------|-------------|----------------|----------|----------------------------|------------|--------------|
| All                                   | 90,411 | 133         | 100.00%        | 100.00%  | 2,241.89                   | 420,307.18 |              |
| CD4+                                  | 4,514  | 133         | 4.99%          | 4.99%    | 18,112.84                  | 87,837.91  |              |

| Plot 3: B01 Naive Memory panel 561438<br>Gated on (CD4+ in all) | Count | Volume (µL) | % of This Plot | % of All | Mean FL1 CD45RA FITC-A | Mean FL4 CD197 AF 647-A | CV I |
|---|-------|-------------|----------------|----------|------------------------|-------------------------|------|
| This Plot   | 4,514 | 133         | 100.00%        | 4.99%    | 8,411.61               | 5,388.40                |      |
| Central Memory  | 1,161 | 133         | 25.72%         | 1.28%    | 1,191.51               | 5,723.84                |      |
| Naive   | 1,710 | 133         | 37.88%         | 1.89%    | 20,352.13              | 8,850.47                |      |
| Effector Memory   | 1,624 | 133         | 35.98%         | 1.80%    | 924.83                 | 1,541.82                |      |
| Q1-LR   | 19    | 133         | 0.42%          | 0.02%    | 14,874.37              | 2,087.11                |      |



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# Ready to Acquire Data

**BD Accuri™ C6**

Collect Analyze Statistics Batch Analysis

**B01** Naive Memory panel 561438

|   |    |    |    |    |    |    |    |    |    |     |     |     |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  | 11  | 12  |
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

**Run Settings**

Run Unlimited  
 Run with Limits

2000 events in CD4+

Do not collect events outside Lymphocytes

**Fluidics**

Slow  Medium  Fast

Flow Rate 86 µL/min  
Core Size 22 µm

Custom

Flow Rate 14 µL/min  
Core Size 10 µm

**Threshold**

Set Threshold  
350,000 on FSC-H  
None

**ADD to B01**

Set Color Compensation

**Last Run**

|                | Cumulative | Delete Events                        | show warning                        |
|----------------|------------|--------------------------------------|-------------------------------------|
| 0 Events       | 90,411     | <input checked="" type="radio"/> All | <input checked="" type="checkbox"/> |
| 0:00.0 Time    | 2:01.5     | <input type="radio"/> Outside        | Lymphocytes                         |
| 0 Microliters  | 133        |                                      |                                     |
| 0 Events / Sec | 7.43       |                                      |                                     |
| 0 Events / µL  | 680        |                                      |                                     |

Data Capacity Used <1% of 96,000,000 Events

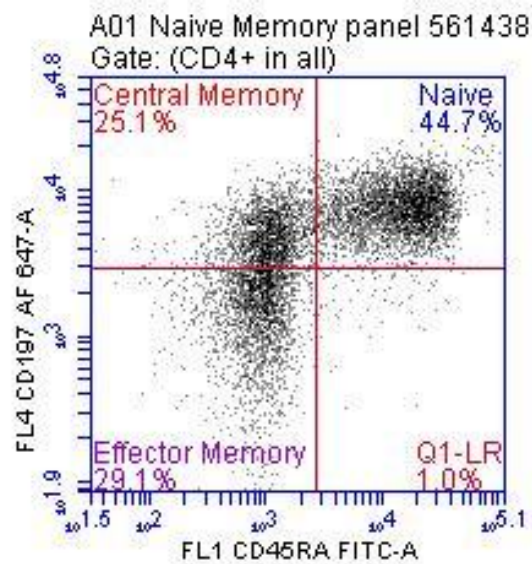
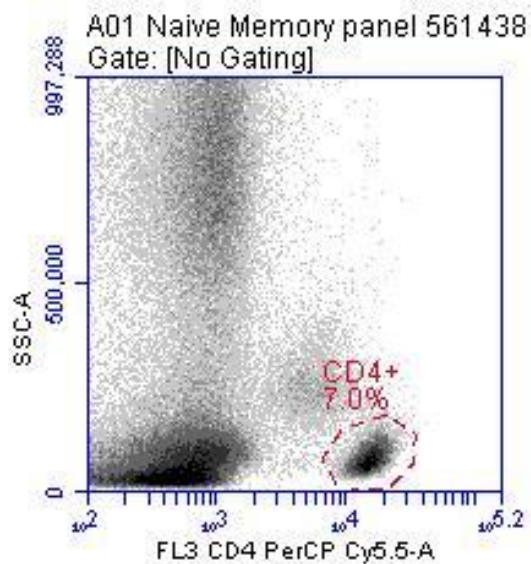
Select plot type to make a new plot.



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# Naïve/Memory T-Cell Data



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# How to Further Optimize a Template: BD Multitest CD45RA/CD45RO/CD3/CD4 (340571)

The screenshot displays the BD Accuri C6 software interface. At the top, there is a menu bar (File, Edit, Display, Instrument, About) and the product name 'BD Accuri™ C6'. Below this are tabs for 'Collect', 'Analyze', 'Statistics', and 'Batch Analysis'. The 'Analyze' tab is active, showing a grid of 12 sample wells (A1-H12) with 'D6' selected in well D6. A status bar indicates 'C6 is connected and ready.' Below the grid are 'Run Settings' and 'Fluidics' sections. The 'Run Settings' section includes options for 'Run Unlimited' or 'Run with Limits', 'Flow Rate' (66 µL/min), 'Core Size' (22 µm), and 'Threshold' (None). The 'Fluidics' section includes options for 'Slow', 'Medium', or 'Fast' flow rate, 'Flow Rate' (14 µL/min), and 'Core Size' (10 µm). A large 'RUN' button is visible. The main area contains five flow cytometry plots: Plot 1 (SSC-A vs FSC-A) showing 'Lymphocytes 6.5%', Plot 2 (SSC-A vs FL3 CD3 PerCP-A) showing 'CD3+ 3.7%', Plot 3 (FL4 CD4 APC-A vs FL1 CD45RA FITC-A) showing 'Naive 11.5%', Plot 4 (FL4 CD4 APC-A vs FL2 CD45RO PE-A) showing 'Memory 49.1%', and Plot 5 (FL4 CD4 APC-A vs FL1 CD45RA FITC-A) showing 'Naive 11.5%'. Below the plots are instructions: 'Select plot type to make a new plot.' and 'Select plot type to make a new plot.' At the bottom, there are three data tables for the plots.

| Plot 1: D06 Multitest 340571 4 color | Count   | Volume ( µL) | % of This Plot | % of All | Mean FSC-A   | Mean SSC-A | CV FSC-A | CV SSC-A | Median F |
|--------------------------------------|---------|--------------|----------------|----------|--------------|------------|----------|----------|----------|
| All                                  | 130,063 | 87           | 100.00%        | 100.00%  | 608,351.90   | 110,126.28 | 117.06%  | 214.07%  |          |
| Lymphocytes                          | 8,517   | 87           | 6.55%          | 6.55%    | 1,013,316.42 | 84,868.57  | 23.65%   | 39.44%   |          |

| Plot 2: D06 Multitest 340571 4 color | Count   | Volume ( µL) | % of This Plot | % of All | Mean FL3 CD3 PerCP-A | Mean SSC-A | CV FL3 CD3 PerCP-A |
|--------------------------------------|---------|--------------|----------------|----------|----------------------|------------|--------------------|
| All                                  | 130,063 | 87           | 100.00%        | 100.00%  | 982.33               | 110,126.28 | 279.65%            |
| CD3+                                 | 4,787   | 87           | 3.68%          | 3.68%    | 11,875.01            | 82,745.39  | 34.32%             |

| Plot 5: D06 Multitest 340571 4 color | Count | Volume ( µL) | % of This Plot | % of All | Mean FL1 CD45RA FITC-A | Mean FL4 CD4 APC-A | CV FL1 CD45 |
|--------------------------------------|-------|--------------|----------------|----------|------------------------|--------------------|-------------|
| This Plot                            | 4,787 | 87           | 100.00%        | 3.68%    | 2,554.49               | 20,614.70          |             |
| Naive                                | 550   | 87           | 11.49%         | 0.42%    | 5,731.86               | 29,650.44          |             |


Panel:  
**CD3 PerCP**  
**CD4 APC**  
**CD45RA FITC**  
**CD45RO PE**



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# How to Optimize a Template: Zoom Tool

File Edit Display Instrument About

**BD CSampler™** 

Manual Collect Auto Collect Analyze Statistics Batch Analysis

Plate Type: 24 tube rack

Plate Name:

A01 Multitest 340571 4 color

|    |    |    |    |    |    |    |    |    |     |     |     |
|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

CS is connected and ready.

**Run Settings**

Run Unlimited  Run with Limits

0 events in   Min 30 Sec

Do not collect events outside

**Fluidics**

Slow  Medium  Fast

Flow Rate 66 µL/min Core Size 22 µm

Custom Flow Rate 14 µL/min Core Size 10 µm

**Threshold**

80,000 on FSC-H None

**Last Run** **Cumulative**   show warning

0 Events 130,063  All

0:00:0 Time 1:19.2  Outside

0 Microliters 87  Data Capacity Used 1% of 24,000,000 Events

Plot 1: A01 Multitest 340571 4 color (GATE) [No Gating] Lymphocytes 6.5%

Plot 2: A01 Multitest 340571 4 color (GATE) [No Gating] CD3 3%

Plot 5: A01 Multitest 340571 4 color (GATE) (CD3+ in all) Naive 11.3%

Plot 6: A01 Multitest 340571 4 color (GATE) (CD3+ in all) Memory 49.1%

Plot 7: A01 Multitest 340571 4 color (GATE) [No Gating] CD3+CD4+ 2.7%

Select plot type to make a new plot.

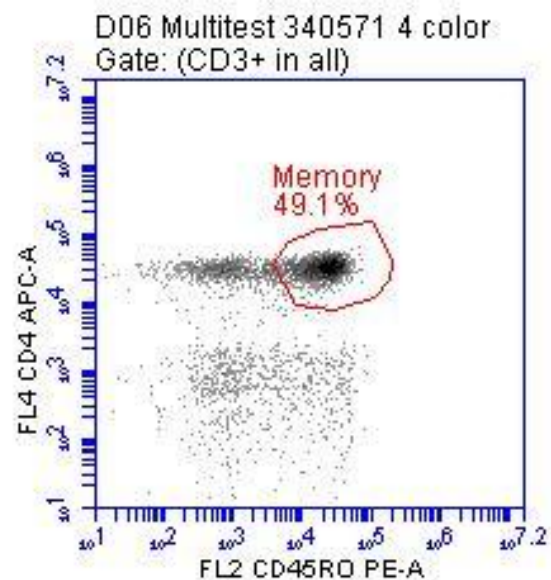
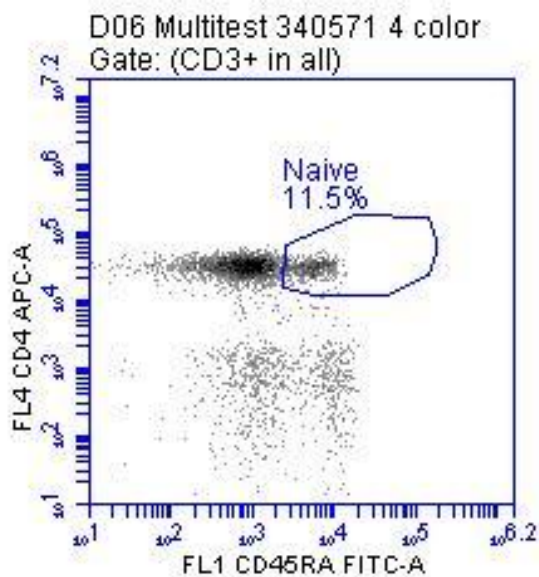
| Plot 3: A01 Multitest 340571 4 color | Count   | Volume (µL) | % of This Plot | % of All | Mean FL3 CD3 PerC-A | Mean FL4 CD4 APC-A | CV FL3 CD3 Pe |
|--------------------------------------|---------|-------------|----------------|----------|---------------------|--------------------|---------------|
| All                                  | 130,063 | 87          | 100.00%        | 100.00%  | 982.33              | 1,172.21           | 27            |
| CD3+CD4+                             | 3,495   | 87          | 2.69%          | 2.69%    | 12,114.68           | 29,970.84          | 1             |



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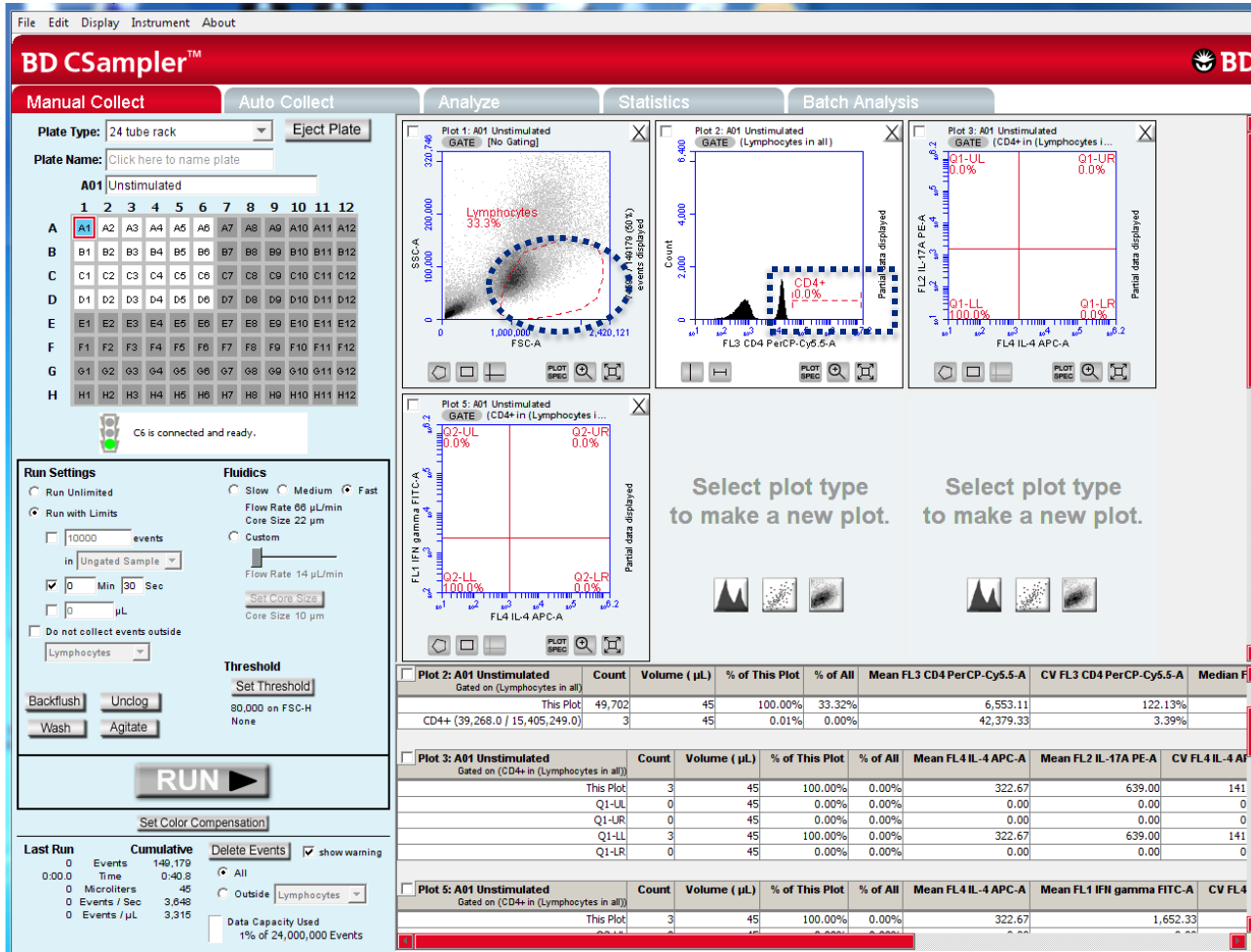


# BD Multitest CD45RA/CD45RO/CD3/CD4 Data



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# How to Optimize a Template for the BD CSampler™ Accessory: Th1/Th2/Th17 Phenotyping Kit (560751)



Panel:  
CD4 PerCP-Cy5.5  
IL-17A PE  
IFN-γ FITC  
IL-4 APC



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# How to Optimize a Template: BD CSampler

File Edit Display Instrument About

## BD CSampler™

Manual Collect Auto Collect Analyze Statistics Batch Analysis

Plate Type: 24 tube rack Eject Plate

Plate Name: Click here to name plate

A01 Unstimulated

|    |    |    |    |    |    |    |    |    |     |     |     |
|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

**Run Settings**

Run Unlimited  Run with Limits

10000 events in Ungated Sample

0 Min 30 Sec

0 μL

Do not collect events outside Lymphocytes

**Fluidics**

Slow  Medium  Fast

Flow Rate 66 μL/min Core Size 22 μm

Custom

Flow Rate 14 μL/min

Set Core Size

Core Size 10 μm

Set Threshold

300,000 on FSC-H

None

Backflush Unclog Wash Agitate

**RUN**

Set Color Compensation

**Last Run**

0 Events 110.572 Cumulative

0.00 Time 0-40.8

0 Microliters 45

0 Events / Sec 2.704

0 Events / μL 2.457

Delete Events  show warning

All

Outside Lymphocytes

Data Capacity Used <1% of 24,000,000 Events

Plot 1: A01 Unstimulated GATE (No Gating)

SSC-A

FSC-A

Lymphocytes 64.9%

66288 / 116272 (60%) events displayed

Plot 2: A01 Unstimulated GATE (Lymphocytes in all)

Count

FL3 CD4 PerCP-Cy5.5-A

3%

Partial data displayed

Plot 3: A01 Unstimulated GATE (CD4+ in Lymphocytes i...)

FL2 IL-17A PE-A

FL4 IL-4 APC-A

Q1-UL 0.0%

Q1-UR 0.0%

Q1-LR 0.1%

Q1-LL 99.9%

Partial data displayed

Select plot type to make a new plot.

| Plot 2: A01 Unstimulated Gated on (Lymphocytes in all) | Count  | Volume (μL) | % of This Plot | % of All | Mean FL3 CD4 PerCP-Cy5.5-A | CV FL3 CD4 PerCP-Cy5.5-A | Median F |
|--|--------|-------------|----------------|----------|----------------------------|--------------------------|----------|
| This Plot  | 71,783 | 45          | 100.00%        | 64.92%   | 7,180.20                   | 162.29%                  |          |
| CD4+ (3,308.0 / 15,405,249.0)                          | 29,667 | 45          | 41.33%         | 26.83%   | 16,580.21                  | 80.40%                   |          |

| Plot 3: A01 Unstimulated Gated on (CD4+ in (Lymphocytes in all)) | Count  | Volume (μL) | % of This Plot | % of All | Mean FL4 IL-4 APC-A | Mean FL2 IL-17A PE-A | CV FL4 IL-4 AP |
|--|--------|-------------|----------------|----------|---------------------|----------------------|----------------|
| This Plot  | 29,667 | 45          | 100.00%        | 26.83%   | 582.95              | 360.59               | 13,796         |
| Q1-UL  | 6      | 45          | 0.02%          | 0.01%    | 183.17              | 8,644.17             | 73             |
| Q1-UR  | 1      | 45          | 0.00%          | 0.00%    | 1,646,264.00        | 1,606.00             | 0              |
| Q1-LL  | 29,644 | 45          | 99.92%         | 26.81%   | 62.99               | 358.85               | 286            |
| Q1-LR  | 16     | 45          | 0.05%          | 0.01%    | 861,232.50          | 401.56               | 386            |

| Plot 5: A01 Unstimulated Gated on (CD4+ in (Lymphocytes in all)) | Count  | Volume (μL) | % of This Plot | % of All | Mean FL4 IL-4 APC-A | Mean FL1 IFN gamma FITC-A | CV FL4 |
|--|--------|-------------|----------------|----------|---------------------|---------------------------|--------|
| This Plot  | 29,667 | 45          | 100.00%        | 26.83%   | 582.95              | 932.94                    |        |




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# How to Optimize a Template: BD CSampler

File Edit Display Instrument About

**BD CSampler™** 


Manual Collect **Auto Collect** Analyze Statistics Batch Analysis

Plate Type: 24 tube rack

Plate Name:

Ctrl-click to view sample settings. [Select All](#) [Deselect All](#)

|   |    |    |    |    |    |    |    |    |    |     |     |     |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  | 11  | 12  |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

 CS is connected and ready.

**Run Limits**

in   Min  S

Do not collect events outside   less than

**Wash Settings**

**Agitate Plate**

None every  Well(s)  Run Horizontally

None every  Min  Run Vertically

**Fluidics**

Flow Rate: 14 µL/min  
Pore Size: 10 µm

**Set Threshold**

Delete events on  less than  (Minimum=10)

less than

| Well | Sample Name  | Rename FL1         | Rename FL2    | Rename FL3          | Rename FL4   | Notes |
|------|--------------|--------------------|---------------|---------------------|--------------|-------|
| A01  | Unstimulated | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A02  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A03  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A04  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A05  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A06  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B01  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B02  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B03  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B04  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B05  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B06  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C01  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C02  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C03  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C04  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C05  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C06  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D01  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D02  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D03  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D04  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D05  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D06  |              | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |



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# How to Optimize a Template: BD CSampler

File Edit Display Instrument About

## BD CSampler™

Manual Collect **Auto Collect** Analyze Statistics Batch Analysis

Plate Type: 24 tube rack Eject Plate

Plate Name: [Click here to name plate](#)

[Select All](#)  
[Deselect All](#)

Ctrl-click to view sample settings.

|   |    |    |    |    |    |    |    |    |    |     |     |     |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  | 11  | 12  |
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

**Run Limits**

0000 events

in CD4+ in (Lymph...)

0 μL

Do not collect events outside Lymphocytes

**Wash Settings**

None

Apply Settings Remove Settings

**Agitate Plate**

None every 1 Well(s)  Run Horizontally

None every 1 Min  Run Vertically

OPEN RUN DISPLAY

| Well | Sample Name       | Rename FL1         | Rename FL2    | Rename FL3          | Rename FL4   | Notes |
|------|-------------------|--------------------|---------------|---------------------|--------------|-------|
| A01  | Unstimulated      | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A02  | PMA+Ionomycin     | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A03  | FITC single       | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A04  | APC single        | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A05  | PE single         | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| A06  | PerCpCy5.5 single | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B01  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B02  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B03  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B04  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B05  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| B06  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C01  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C02  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C03  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C04  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C05  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| C06  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D01  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D02  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D03  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
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| D05  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |
| D06  |                   | FL1 IFN gamma FITC | FL2 IL-17A PE | FL3 CD4 PerCP-Cy5.5 | FL4 IL-4 APC |       |

Fluidics: Flow Rate: 86 μL/min, Core Size: 22 μm

Set Threshold: Delete events on (Minimum=10)  
FSC-H less than 900000  
less than 0

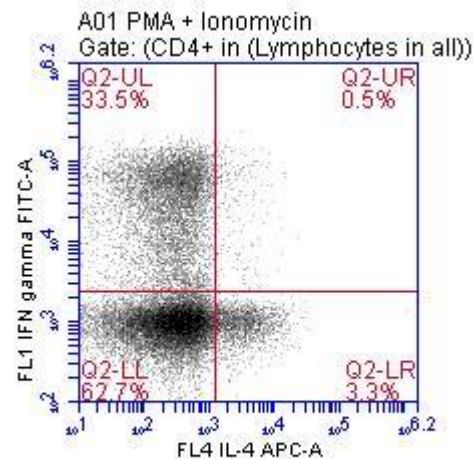
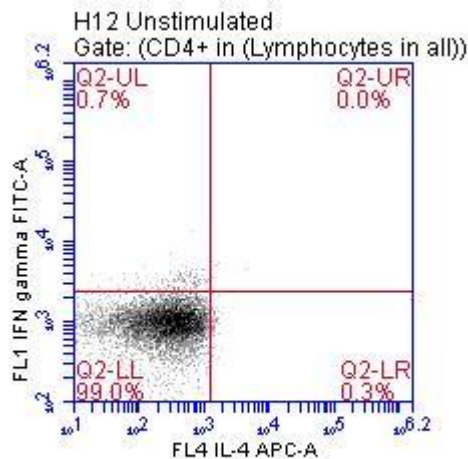
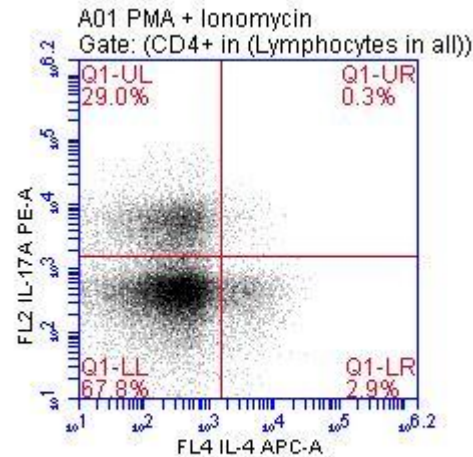
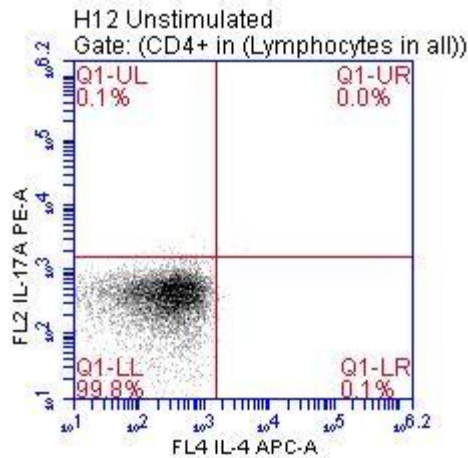


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# BD Pharmingen™ Human Th1/Th2/Th17 Phenotyping Kit Data

Unstimulated

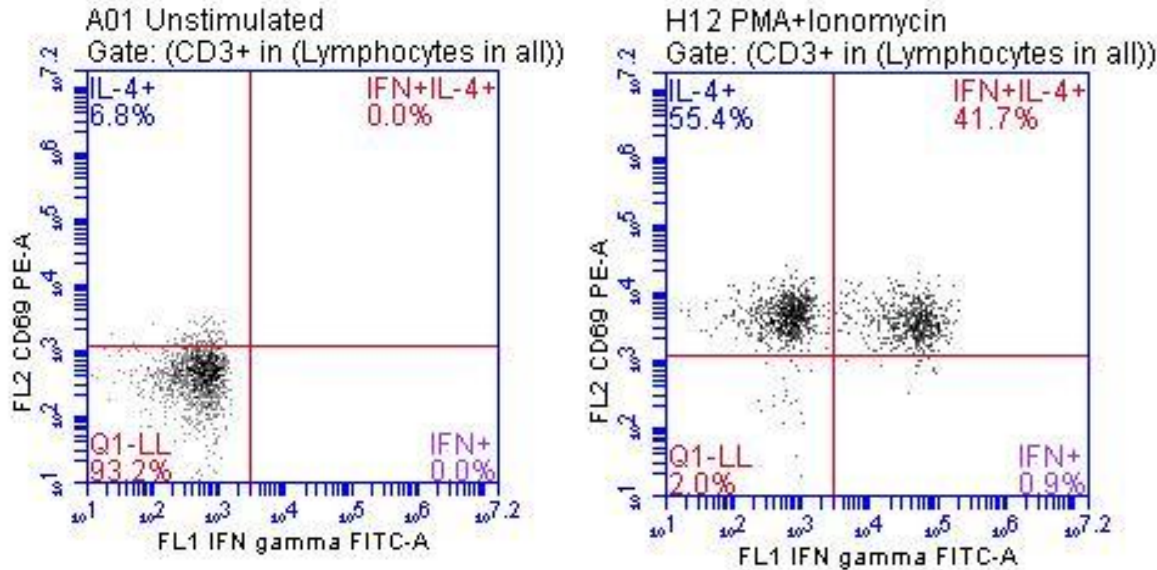
Stimulated



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# Application-Specific Templates: BD FastImmune™ Kits (346048 and 340456)

- Detection of intracellular T-cell cytokines using preconfigured kits. Available with or without CD69 (activation marker).



**Panel:**  
CD8 PerCP-Cy5.5  
CD3 APC  
IFN- $\gamma$  FITC  
CD69 PE



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# Where to Find the Templates

- [www.bdbiosciences.com/go/templates](http://www.bdbiosciences.com/go/templates)



| Category     | Product Information Sheet               | Brand              | Kit                                       | Cat. No. | Template |
|--------------|---|--------------------|---|----------|----------|
| Cell Biology | Apoptosis Kits and Templates            | BD Pharmingen™     | Annexin V FITC Apoptosis Detection Kit II | 556570   | Download |
|              |   | BD Pharmingen™     | Annexin V PE Apoptosis Detection Kit I    | 559763   | Download |
|              |   | BD™                | MitoScreen (JC-1) Kit                     | 551302   | Download |
|              |   | BD Pharmingen™     | Caspase-3 PE Assay Kit                    | 550914   | Download |
|              | Cell Cycle and DNA Kits and Templates   | BD Cycletest™ Plus | DNA Reagent Kit                           | 340242   | Download |
|              |   | BD Pharmingen™     | FITC BrdU Flow Kit                        | 559619   | Download |
|              |   | BD Pharmingen™     | APC BrdU Flow Kit                         | 552598   | Download |
| Immunology   | Naïve/Memory T- Cell Kits and Templates | BD Multitest™      | CD45RA/CD45RO/CD3/CD4                     | 340571   | Download |
|              |   | BD Multitest™      | CD45RA/CD62L/CD3/CD4                      | 340977   | Download |
|              |   | BD Pharmingen™     | Human Naïve/Memory T Cell Panel           | 561438   | Download |
|              | Intracellular T-Cell Kits and Templates | BD Pharmingen™     | Th1/Th2/Th17 Phenotyping Kit              | 560751   | Download |
|              |   | BD FastImmune™     | IFN-γ/CD69/CD8/CD3 Kit                    | 346048   | Download |
|              |   | BD FastImmune™     | IFN-γ/IL4 Kit                             | 340456   | Download |

# BD Templates Available for Free on the Web

**EXISTING**

6, 8-Peak Bead  
BD™ CBA  
Water Quality

**NEW**

Proliferation: BrdU  
Apoptosis: Annexin V  
Apoptosis: Caspase-3  
DNA Analysis  
BD™ MitoScreen  
Stem Cell Flow Kits  
T-Cell Cytokines  
Naïve/memory T Cells



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# Outline

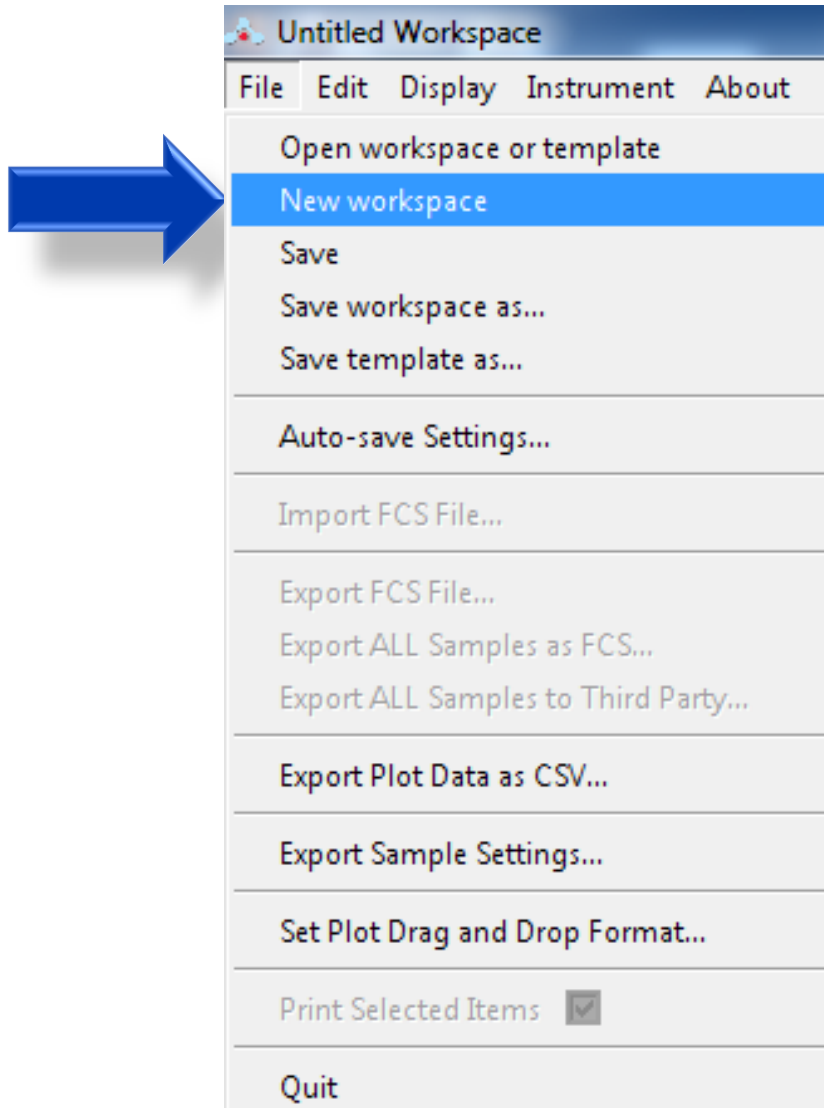
---

- Background: Immunophenotyping
- Introduction to the BD Accuri™ C6 Flow Cytometer
- BD Kits and Templates
- BD Templates on the Web
- **How to Create Your Own Templates**
- Coming Soon....



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# How to Create Your Own Templates

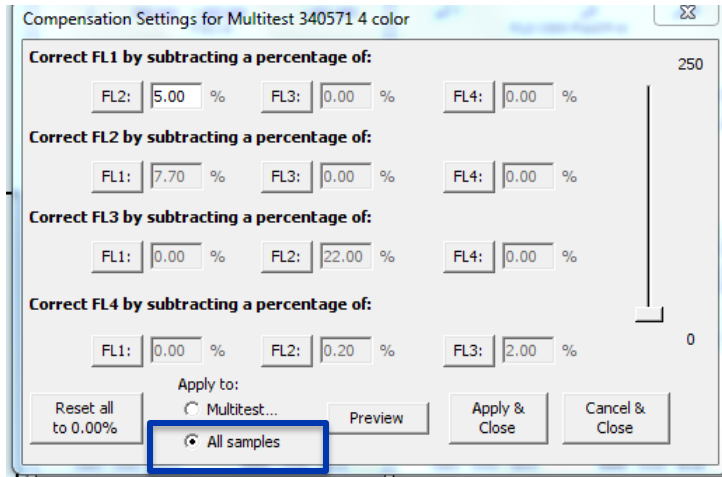


1. Open a new workspace or existing workspace.



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# How to Create Your Own Templates



Compensation Settings for Multitest 340571 4 color

Correct FL1 by subtracting a percentage of: 250

FL2: 5.00 % FL3: 0.00 % FL4: 0.00 %

Correct FL2 by subtracting a percentage of:

FL1: 7.70 % FL3: 0.00 % FL4: 0.00 %

Correct FL3 by subtracting a percentage of:

FL1: 0.00 % FL2: 22.00 % FL4: 0.00 %

Correct FL4 by subtracting a percentage of:

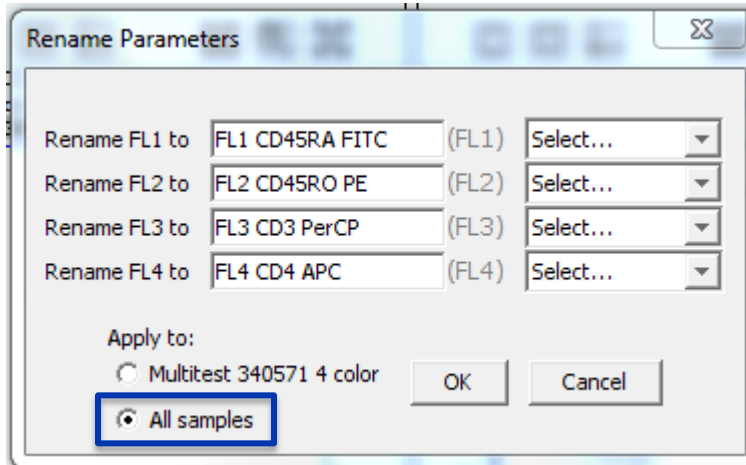
FL1: 0.00 % FL2: 0.20 % FL3: 2.00 %

Apply to:

Multitest...  All samples

Reset all to 0.00% Preview Apply & Close Cancel & Close

2. Collect samples, optimize plots and run criteria to desired settings.
3. If desired, input compensation, parameter names, and threshold settings and click Apply to: All samples.



Rename Parameters

Rename FL1 to FL1 CD45RA FITC (FL1) Select...

Rename FL2 to FL2 CD45RO PE (FL2) Select...

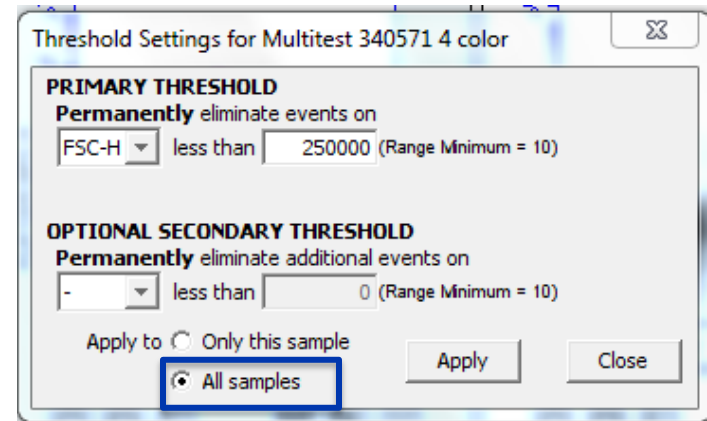
Rename FL3 to FL3 CD3 PerCP (FL3) Select...

Rename FL4 to FL4 CD4 APC (FL4) Select...

Apply to:

Multitest 340571 4 color  All samples

OK Cancel



Threshold Settings for Multitest 340571 4 color

**PRIMARY THRESHOLD**  
Permanently eliminate events on  
FSC-H less than 250000 (Range Minimum = 10)

**OPTIONAL SECONDARY THRESHOLD**  
Permanently eliminate additional events on  
- less than 0 (Range Minimum = 10)

Apply to  Only this sample  All samples

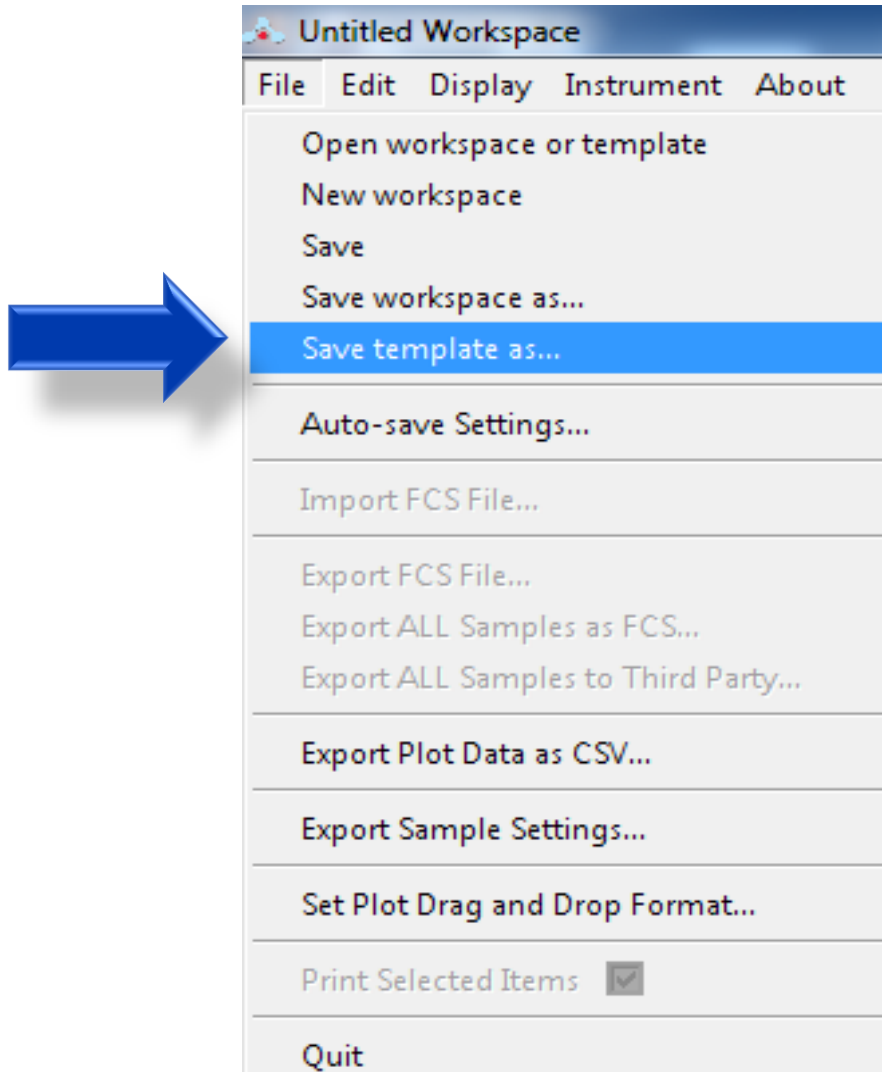
Apply Close



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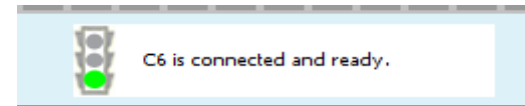
# How to Create Your Own Templates



4. Select File > Save Template As.

5. Save the template with a new name.

Note: Creating templates works best at the cytometer workstation, when the BD Accuri C6 is powered on.



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# Important Points to Remember

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- Customize templates to fit your sample type and workflow.
- Verify all settings, including thresholds and compensation.
- When using the BD CSampler, copy desired settings to the Auto Collect tab.
- For best results, create templates when the software is connected to the cytometer.



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# Outline

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- Background: Immunophenotyping
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- How to Create Your Own Templates
- **Coming Soon....**



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# BD Templates Coming Soon to the Web.....

COMING SOON

NEW

Tregs  
Mouse Lymphocytes  
Rat Lymphocytes  
Mouse Tregs  
Mouse T-Cell  
Cytokines  
Counting and Viability  
of Bacteria  
Mesenchymal Stem  
Cell Kit

Proliferation: BrdU  
Apoptosis: Annexin V  
Apoptosis: Caspase-3  
DNA Analysis  
BD MitoScreen  
Stem Cell Flow Kits  
T-Cell Cytokines  
Naïve/memory T Cells

EXISTING

6, 8-Peak Bead  
BD CBA  
Water Quality



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# Special Promotional Offer

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For a limited time, take advantage of **big savings** and value-added packages for the BD Accuri C6 flow cytometer with our reagent kit discount.

**Get a 40% discount on reagent kits** now through June 2014

*New to BD Accuri?*

**Buy a BD Accuri C6** now and receive an immediate **10% discount** off the purchase price. You'll also be eligible to receive **40% off the list price of all BD Pharmingen reagents** you purchase for use on the BD Accuri C6 for the next 2 years.

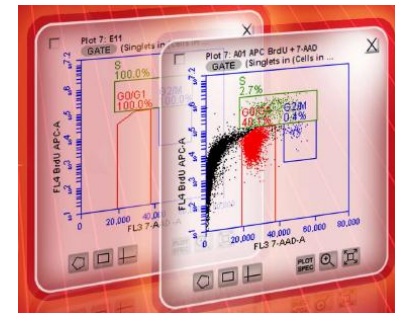
Offer expires June 30<sup>th</sup>, 2014.

Now you can simplify your workflow while dramatically reducing the cost of use of your personal flow cytometer.

[www.bdbiosciences.com/go/templates](http://www.bdbiosciences.com/go/templates)

# Summary

- The **BD Accuri C6** is making it even easier to apply the power of flow cytometry to your research with **free software templates** and **ready-to-go reagent kits** specific to your studies.
- Find out more about how the BD Accuri C6 puts the power of 4-color cell analysis within reach by visiting [www.bdbiosciences.com/go/templates](http://www.bdbiosciences.com/go/templates)



***Flow Cytometry Within Reach.***



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# For more information...

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If you have further questions:

Contact Technical Support (US) at:

877-232-8995, Prompt 3, 2

or email: [ResearchApplications@bd.com](mailto:ResearchApplications@bd.com)

Please visit our BD Accuri resources site at:

[www.bdbiosciences.com/resources/accuri](http://www.bdbiosciences.com/resources/accuri).

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