PN 400270 B1
TECHNICAL NOTE

Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit

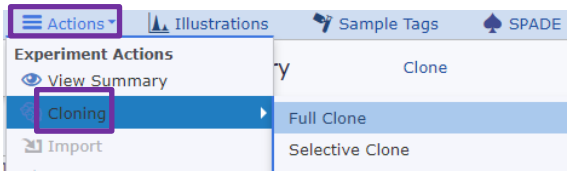
Introduction

The Fluidigm Maxpar® Human Immune Monitoring Panel Kit 201324 Gating Example was developed in collaboration with Cytobank specifically for use with the Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324). This gating strategy is based on the Flow Cytometry Results Data Templates created by the Human Immunology Project Consortium (HIPC) and input from Verity Software House. The gating example provides recommendations for gating peripheral blood mononuclear cell (PBMC) immune populations using markers available in the kit and is available as a Public Experiment in Premium Cytobank.

Accessing the Public Experiment

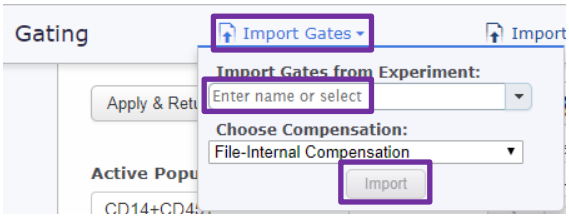
An example analysis for the panel kit, Fluidigm_Maxpar Human Immune Monitoring Panel Kit_201324_Gating Example_v1.0, is available for reference at Premium.Cytobank.org.

Create a Clone of the Public Experiment



The Public Experiment is read-only. To access the Gating tab, create a personal copy by cloning the experiment (available under the Actions tab).

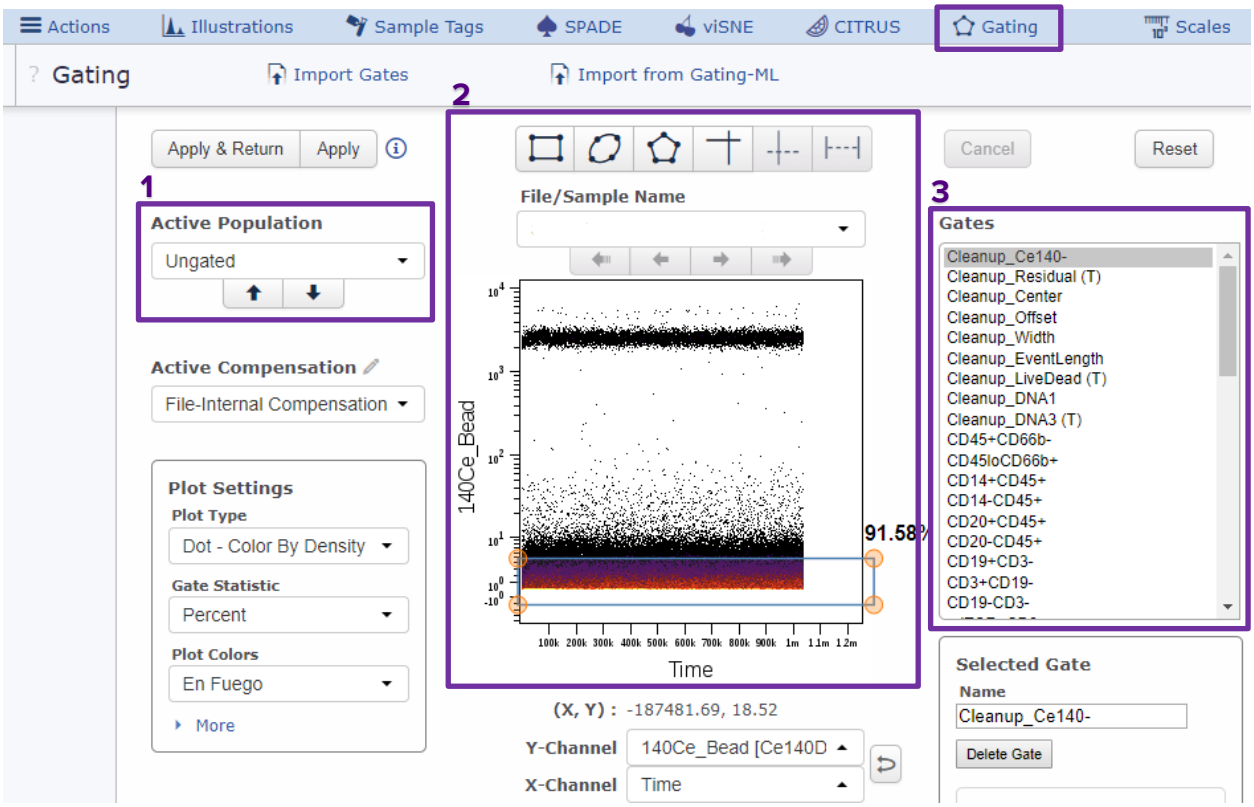
Apply Gating Strategy to New Data



The gating strategy can be applied to new experiments by using the Import Gates function in the Gates tab. Enter the Cytobank experiment number **151947** in the dialog box and click **Import**. This will apply the gating strategy of the selected Cytobank experiment to the files in the new experiment.

Overview of the Cytobank Gating Tab

The Gating Tab within Cytobank is used to create and adjust the gating strategies.



Key features:

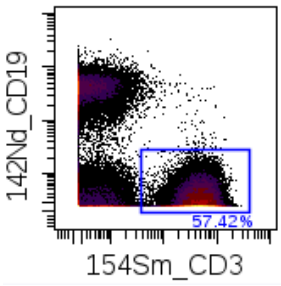
1 **Active Population:** Choose a population to view and gate.

2 **File/Sample Name plot:** View the gate parameters for the active population.

3 **Gates:** Choose a gate name to view and edit.

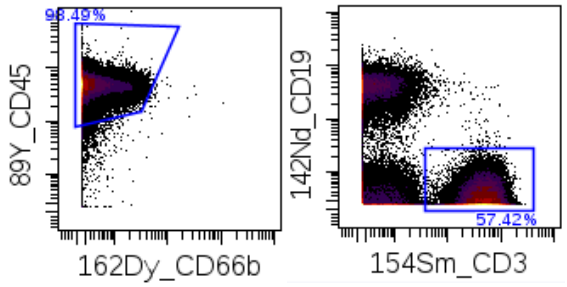
Defining Gates and Populations

In Cytobank, the terms gate and population are not interchangeable.



A gate is a selected region in the plot. Gates are defined on single parameters in histograms or two parameters in bivariate plots, e.g., the CD3+CD19- gate (*Figure 1*).

Figure 1. CD3+CD19- gate.

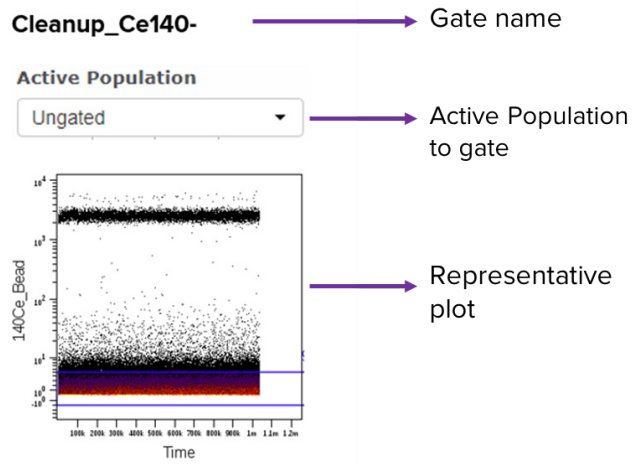


Populations are defined by the gates used to identify each group. For example, T lymphocytes can be broadly defined with two gates: CD45+CD66b-and CD3+CD19-(*Figure 2*).

Figure 2. CD45+CD66b- and CD3+CD19- gates.

Using the Maxpar Human Immune Monitoring Panel Cell Gating Strategy

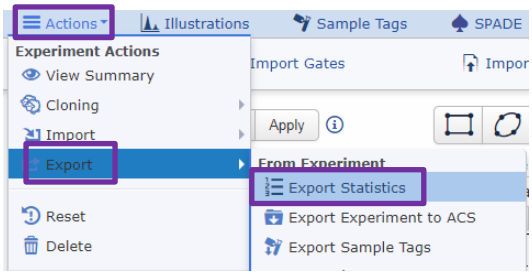
The steps for the cleanup and cell gating are outlined below with the gate name, Active Population, and a representative plot.



To adjust gates:

1. Select the gate name in the Gates box.
2. Select the **Active Population** from the drop-down menu.
3. Review the gate and move to select the appropriate region.

Exporting Statistics from a Cytobank Experiment

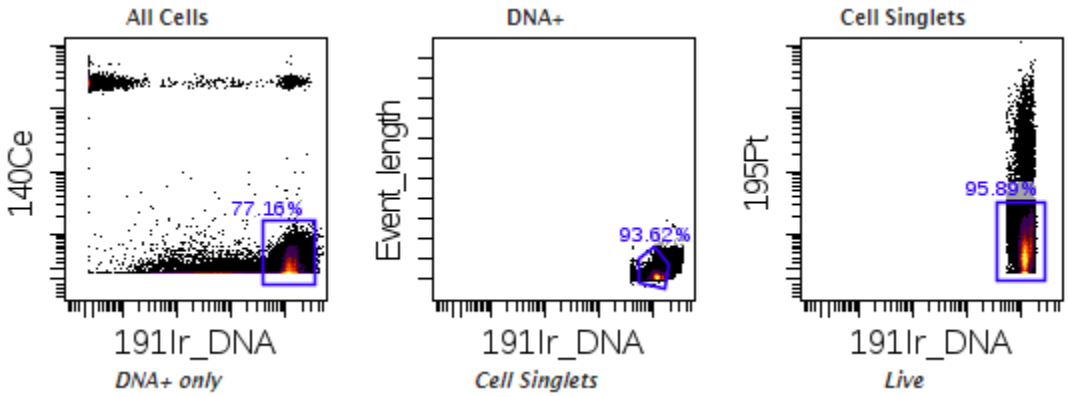


In addition to the gating strategy, statistics (for example, event counts and marker intensities) can be exported using the Export Statistics Tool.

A template for exporting event counts for each population is provided within the Public Experiment (Fluidigm Maxpar Human Immune Monitoring Panel Kit 201324 Gating Example). For more information on Cytobank features go to support.cytobank.org.

Data Cleanup

Prior to cell gating using antibody targets (markers), a cleanup strategy is used to remove debris, normalization beads, doublets, and dead cells. A common cleanup method used in mass cytometry is depicted below. DNA+ events are gated on DNA+Bead- (191Ir+140Ce-), then singlet events are gated using Event Length [191Ir+EventLength(int)], followed by viable cell gating using viability [Cisplatin (191Ir+195Pt-)].



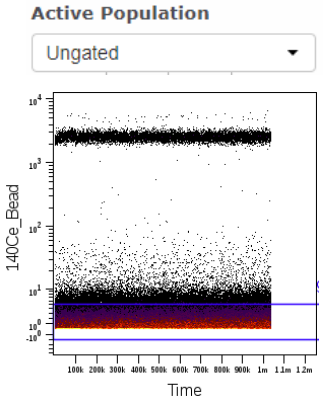
This cleanup strategy was developed by Verity Software House (VSH) and Fluidigm. This method has shown better aggregate and doublet removal than the commonly applied gating strategies. In addition to the common parameters used (DNA, bead, EventLength, viability), this cleanup method uses Gaussian parameters for each event. The Gaussian Discrimination (GD) channels (Center, Width, Offset and Residual) are recorded for each FCS file generated by Helios™.

Cleanup Strategy

Each cleanup parameter is plotted against time. The gates are adjusted to remove aggregates, debris, beads, doublets, and dead cells.

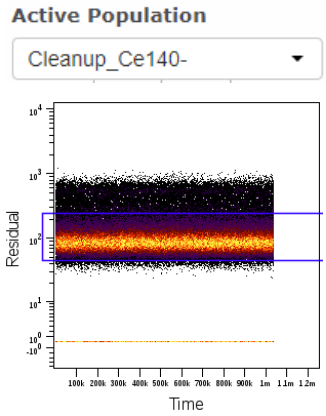
Cleanup Gates

1 Cleanup_Ce140-



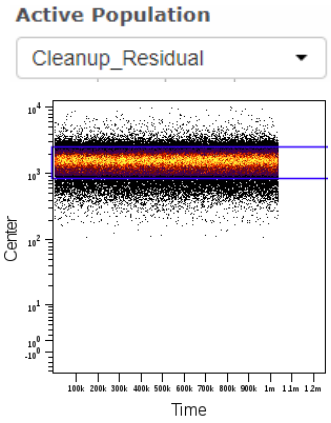
Adjust the Cleanup_Ce140- gate to select the low-intensity events.

2 Cleanup_Residual



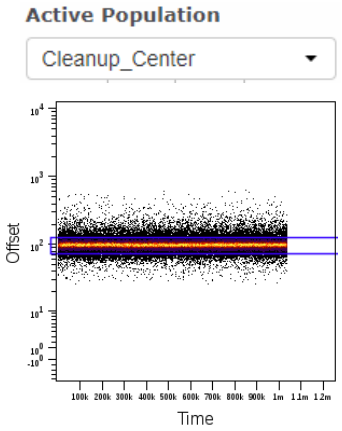
Adjust the Cleanup_Residual gate to select the largest band of events (midrange intensity).

3 Cleanup_Center



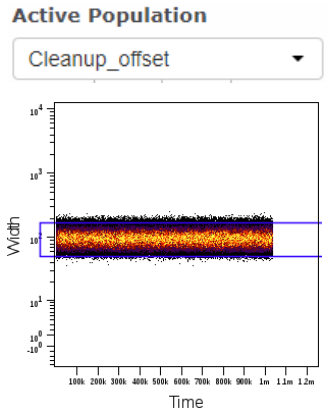
Adjust the Cleanup_Center gate to select the largest band of events (midrange intensity).

4 Cleanup_Offset



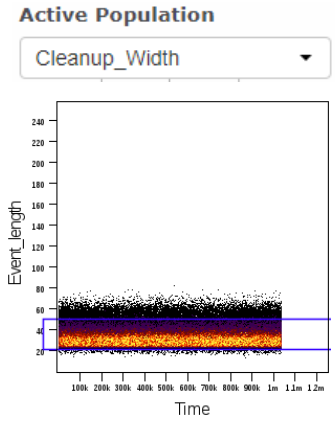
Adjust the Cleanup_Offset gate to select the largest band of events (midrange intensity).

5 Cleanup_Width



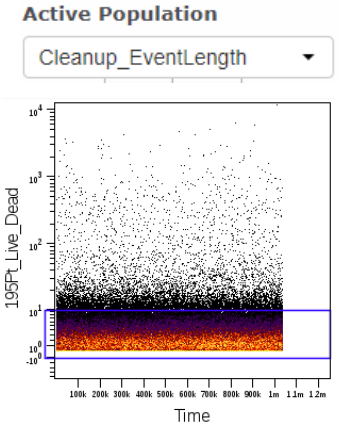
Adjust the Cleanup_Width gate to select the largest band of events (midrange intensity).

6 Cleanup_EventLength



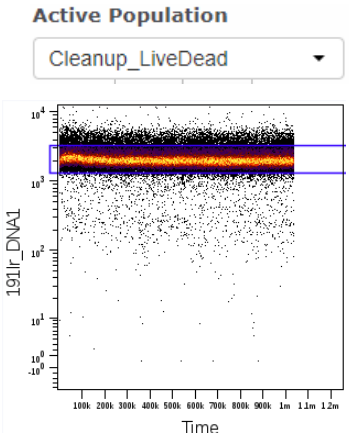
Adjust the Cleanup_EventLength gate to select the largest band of events (low-range intensity).

7 Cleanup_LiveDead



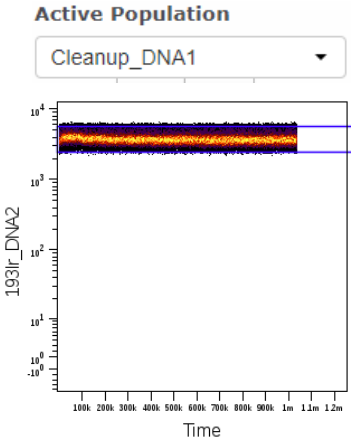
Adjust the Cleanup_LiveDead gate to select the largest band of events (low-range intensity).

8 Cleanup_DNA1



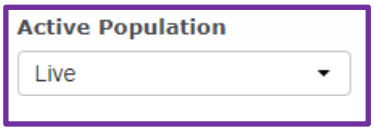
Adjust the Cleanup_DNA1 (191Ir) gate to select the largest band of events (mid-to-high-range intensity).

9 Cleanup_DNA3



Adjust the Cleanup_DNA3 gate to select the largest band of events (mid-to-high-range intensity).

Global Parent Population

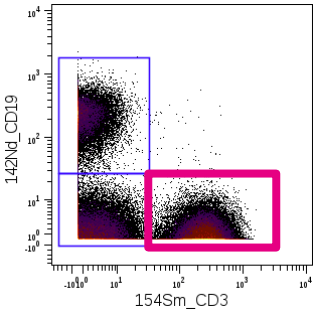


After each of the cleanup gates (Cleanup_Ce140- to Cleanup_DNA3) is applied, the final population comprising all the cleanup gates is labeled the Live population.

This is the global parent population that will be used for subsequent immune cell gating.

Immune Cell Gating

The ability to gate is dependent on the staining intensity of each marker and the resolution between positive and negative populations. Before analysis of critical samples, a preliminary experiment should be done on non-critical samples (see Pilot Experiment 2 in Before You Begin in Maxpar Human Immune Monitoring Panel Kit Protocol). Review Pilot Experiment 2 data for antibody staining quality. Evaluate marker intensities in the pilot experiment that are lower or higher than expected, which may affect the ability to identify populations.



The Maxpar Human Immune Monitoring Panel Kit gating strategy is a manual method that uses bivariate plots to gate on positive and negative regions to identify different immune populations. Gates are adjusted based on the marker expression. For instance, T lymphocytes are gated as CD3+CD19-. The CD19+ population is used as a negative population for CD3+ events. For some markers, a negative population is not available in PBMC. View each gate in each file to ensure correct gating.

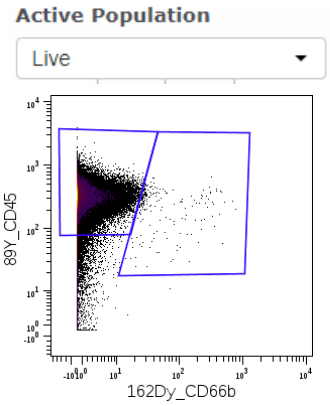
Many gates are used to identify multiple populations. The gates used to define each population, including intermediate populations, are listed in Appendix A: Population Gating Tables.

NOTE For more information, contact your local Fluidigm field application specialist.

Major Cell Populations

The first four gates use lineage markers for immune cells, T cells, B cells, and monocytes. The gates defined in this section will be used for subsequent populations. For instance, the CD14-CD45+ gate will be used for B and T cells.

1 **CD66b vs. CD45:** Lymphocytes, DCs, Monocytes & Granulocytes

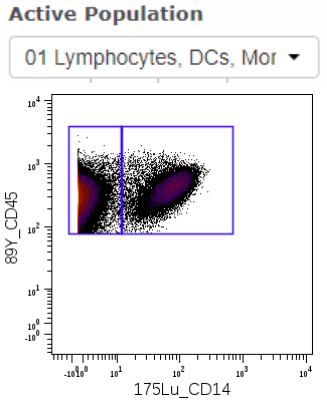


The CD45 vs. CD66b gate is used to remove granulocytes from subsequent cell CD45+ populations:

- *01: CD45+CD66b-: Lymphocytes, DCs, Monocytes*
- *02: CD45+CD66b+: Granulocytes*

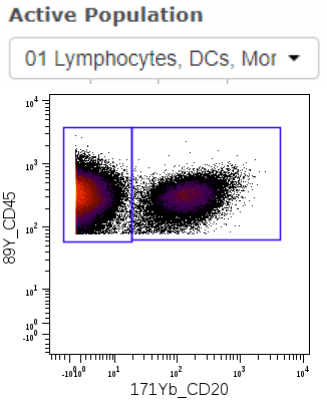
NOTE CD66 expression and granulocyte frequency are sensitive to sample preparation and sample type. PBMC preparations may have few granulocytes.

2 CD14 vs. CD45



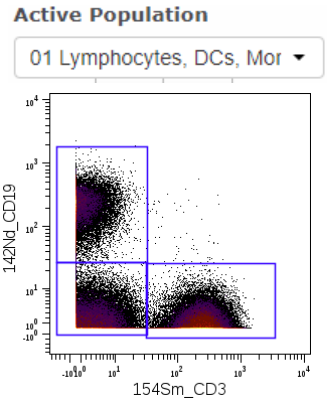
The CD14 vs. CD45 gate is used to set CD14+ and CD14- gates for subsequent cell populations.

3 CD20 vs. CD45



The CD20 vs. CD45 gate is used to set CD20+ and CD20- gates for subsequent cell populations.

4 CD3 vs. CD19

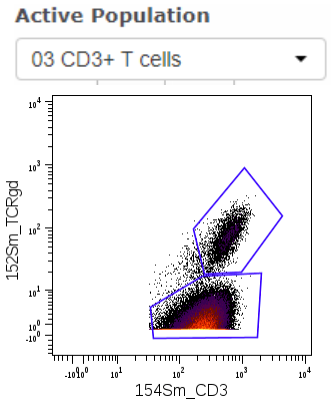


The CD3 vs. CD19 gate is used to set CD3+CD19- and CD3-CD19+ gates for subsequent cell populations.

CD8 and γδ T Cell Subsets

The next five gates distinguish T cell subsets.

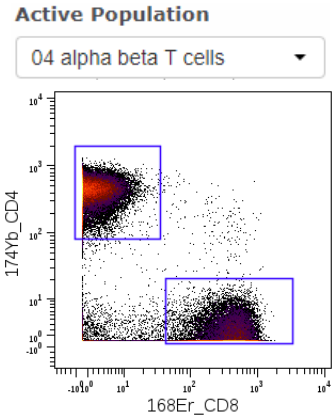
1 CD3 vs. TCRgd



The CD3 vs. TCRgd gate is used to distinguish αβ T cells from γδ T cells:

- *04: TCRgd-CD3+ (ab T cells)*
- *41: TCRgd T cells*

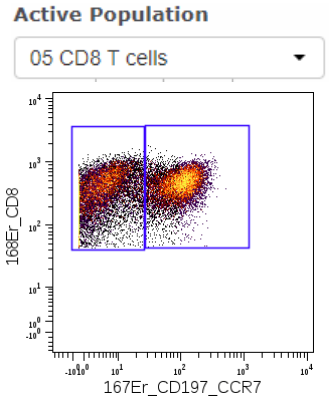
2 CD8 vs. CD4



The CD8 vs. CD4 gate is used to distinguish CD4 T cells from CD8 T cells:

- *05: CD4-CD8+ (CD8 T cells)*
- *11: CD4+CD8- (CD4 T cells)*

3 CCR7 vs. CD8

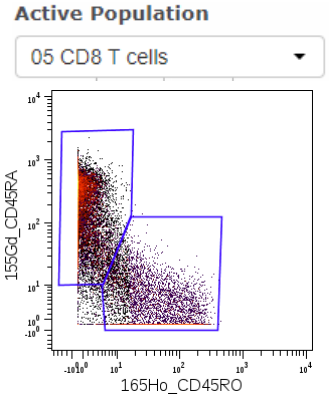


The CCR7 vs. CD8 gate is used as an intermediate population to gate on CD8 subsets:

- *CCR7+CD8+*
- *CCR7-CD8+*

NOTE Spillover may occur between CD8-168Er and CCR7-167Er. The intensity of CD8 and CCR7 should be evaluated in Pilot Experiment 2 on non-critical samples to determine the ability to gate CCR7+CD8+ and CCR7-CD8+ events.

4 CD45RO vs. CD45RA

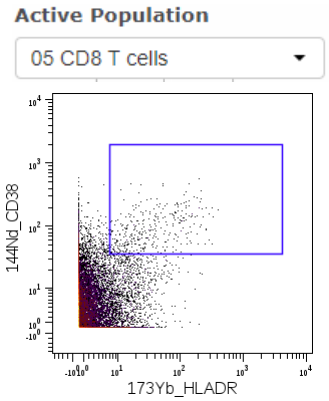


The CD45RO vs. CD45RA gate is used as an intermediate population to gate on CD8 and CD4 subsets:

- *CD45RO+CD45RA-*
- *CD45RA+CD45RO-*

NOTE The CD45RO and CD45RA gate is also used for CD4 T cells. In addition to CD45RO and CD45RA, CD28 is available in the panel to identify T cell subsets.

5 HLADR vs. CD38



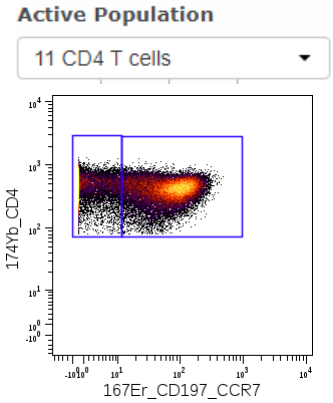
The HLADR vs. CD38 gate is used to identify activated CD8 and CD4 T cells:

- *HLADR+CD38+*

CD4 and Treg Subsets

The next six gates distinguish CD4 and Treg subsets.

1 CCR7 vs. CD4

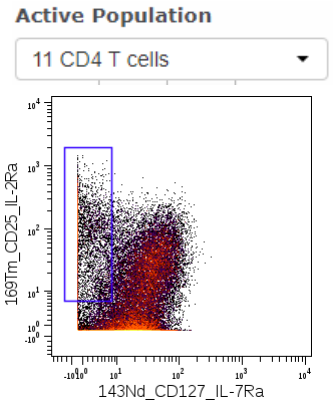


The CCR7 vs. CD4 gate is used as an intermediate population to gate on CD4 subsets:

- *CCR7+CD4+*
- *CCR7-CD4+*

NOTE To aid gating, the CD14+CD45+ population can be used to place the CCR7+ gate.

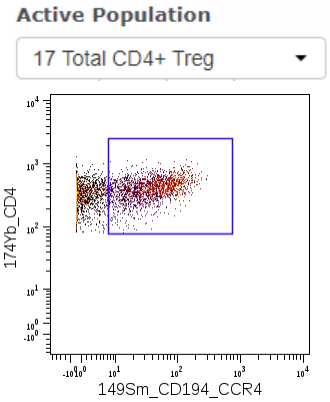
2 CD25 vs. CD127



The CD127 vs. CD25 gate is used as an intermediate population for CD4 Tregs:

- *17 Total CD4+ Treg*

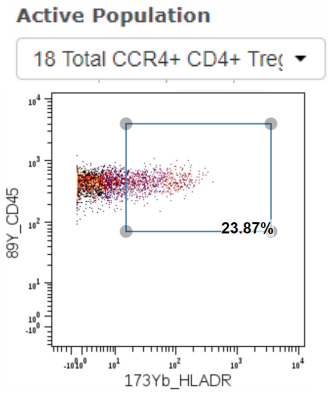
3 CCR4 vs. CD4



The CCR4 vs. CD4 gate is used to identify CCR4+ Tregs:

- *18 Total CCR4+CD4+ Treg*

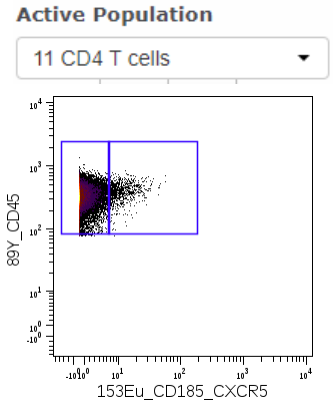
4 HLADR vs. CD45



The HLADR vs. CD45 gate is used to identify activated Tregs:

- *20 Total CCR4+CD4+ Treg Activated*

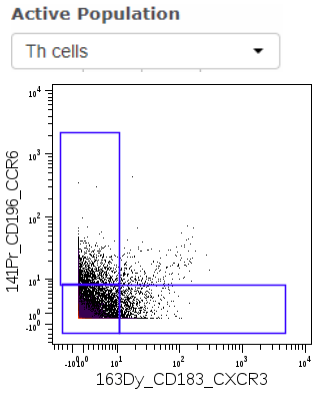
5 CXCR5 vs. CD45



The CXCR5 vs. CD45 gate is used to identify Th subsets:

- *Th: CXCR5+ CD45+*

6 CXCR3 vs. CCR6



The CXCR3 vs. CCR6 gate is used to identify Th subsets:

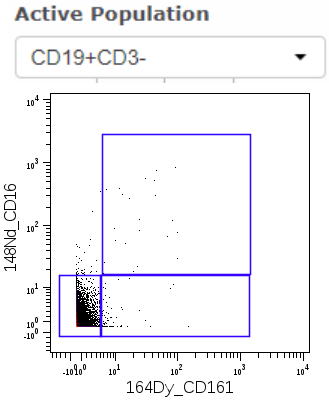
- *22: CXCR3+CCR6- Th1*
- *23: CXCR3-CCR6- Th2*
- *24: CXCR3-CCR6+ Th17*

NOTE Chemokine receptor expression is sensitive to sample type, preparation, and treatment. The intensities of CXCR3, CCR6, and CXCR5 should be evaluated in Pilot Experiment 2 on non-critical samples to determine the ability to gate CD4 Th subsets.

B Cell Subsets

The next four gates distinguish B cell subsets.

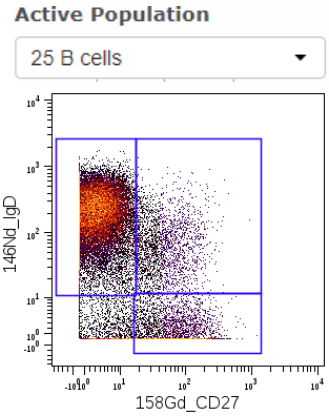
1 CD16 vs. CD161



The CD161 vs. CD16 gate is used to remove aggregates from the B cell population:

- *25: CD161-CD16- (B cells)*

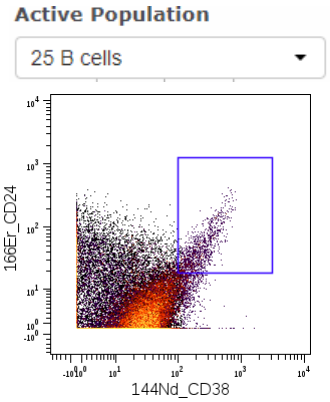
2 CD27 vs. IgD



The CD27 vs. IgD gate is used to identify B cell subsets:

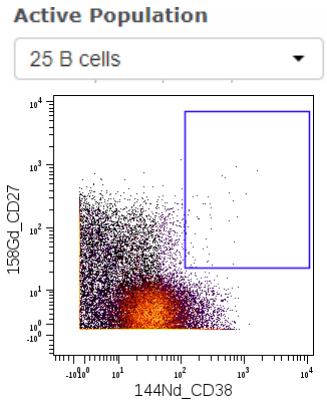
- *26: IgD+CD27- (Naïve B cells)*
- *27: IgD-CD27+ (Memory B cells)*
- *28: IgD+Cd27+ (Memory Resting B cells)*

3 CD38 vs. CD24



The CD38 vs. CD24 gate is used to identify transitional B cells (*29*).

4 CD38 vs. CD27

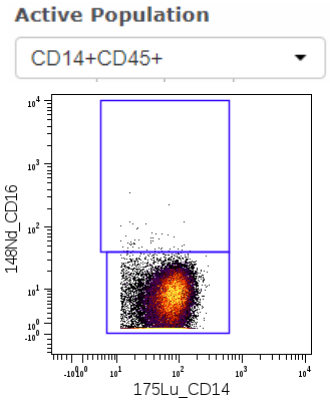


The CD38 vs. CD27 gate is used to identify plasmablasts (*30*).

Monocytes

The next gate distinguishes monocyte subsets.

1 CD14 vs. CD16



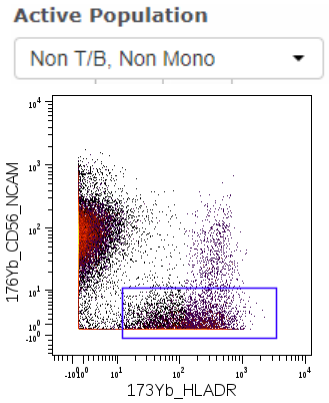
The CD14 vs. CD16 gates is used to identify subsets of monocytes:

- *32: CD16+ (Non-classical Monocytes)*
- *33: CD16- (Classical Monocytes)*

Dendritic Cells (DCs)

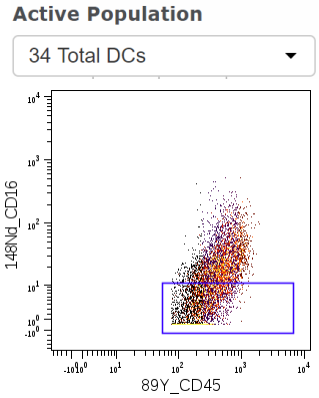
The next three gates distinguish DCs subsets.

1 HLADR vs. CD56



The HLADR vs. CD56 gate is used to identify total DCs (*34*).

2 CD45 vs. CD16

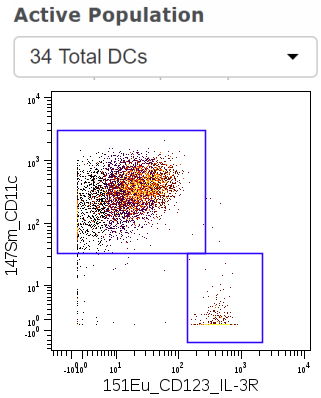


The CD45 vs. CD16 gate is used for DC subsets:

- *CD16-CD45+*

NOTE To aid gating, CD19+CD3- can be used to identify the CD16- population.

3 CD123 vs. CD11c



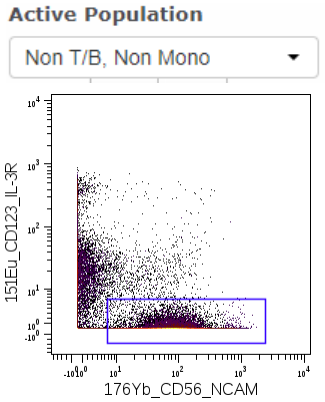
The CD123 vs. CD11c gate is used for DC subsets:

- *35: CD123+CD11c-(pDC)*
- *36: CD123-CD11c+ (mDC)*

Natural Killer Cells (NK Cells)

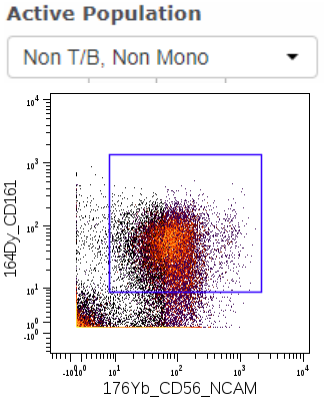
The next three gates distinguish NK cell subsets. NK cells are defined as CD56+CD123-CD161+, which requires two gates: 1) CD56 vs. CD123, and 2) CD56 vs. CD161.

1 CD56 vs. CD123



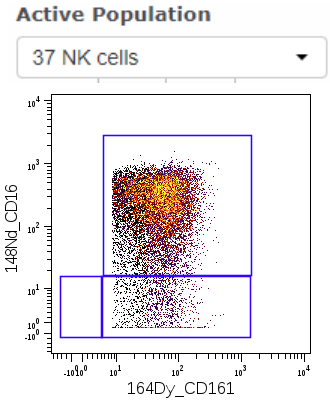
The CD56 vs. CD123 gate is used to identify NK cells.

2 CD56 vs. CD161



The CD56 vs. CD161 gate is used to identify NK cells *(37)*.

3 CD16 vs. CD161



The CD161 vs. CD16 gate is used to identify NK cell subsets:

- *38: CD16-CD161+ (CD16- NK cells)*
- *39: CD16+CD161+ (CD16+ NK cells)*

Considerations

- Marker intensities may vary across different samples. To adjust the gate for each specific file and/or population, use the Gate Tailoring box below the Gates box. For instance, the CD45RA vs. CD45RO gate can be adjusted for CD4 and CD8 T cells.
- Gates can be added and removed for each population in the Population Manager.
- For more information on Cytobank features go to support.cytobank.org.

Appendix A: Population Gating Tables

Table 1. Gates used to distinguish immune cell populations stained by the Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324).

Immune Populations	Gates	Immune Populations	Gates
01 Lymphocytes, DCs, monocytes	CD45+CD66b-	**08** CD8 T cells, effector memory (EM)	CD45+CD66b-
02 Total Granulocytes	CD45loCD66b+		CD3+CD19-
03 CD3+ T cells	CD45+CD66b-		CD20-CD45+
	CD3+CD19-		CD14-CD45+
	CD20-CD45+		gdTCR-CD3+
	CD14-CD45+		CD8+CD4-
04 alpha beta T cells	CD45+CD66b-		CCR7-CD8+
	CD3+CD19-		CD45RA-CD45RO+
	CD20-CD45+	**09** CD8 T cells, terminal effector (TE)	CD45+CD66b-
	CD14-CD45+		CD3+CD19-
	gdTCR-CD3+		CD20-CD45+
05 CD8 T cells	CD45+CD66b-		CD14-CD45+
	CD3+CD19-		gdTCR-CD3+
	CD20-CD45+		CD8+CD4-
	CD14-CD45+		CCR7-CD8+
	gdTCR-CD3+		CD45RA+CD45RO-
	CD8+CD4-	**10** CD8 T cells, activated	CD45+CD66b-
06 CD8 T cells, naive	CD45+CD66b-		CD3+CD19-
	CD3+CD19-		CD20-CD45+
	CD20-CD45+		CD14-CD45+
	CD14-CD45+		gdTCR-CD3+
	gdTCR-CD3+		CD8+CD4-
	CD8+CD4-		HLADR+CD38+
	CCR7+CD8+	**11** CD4 T cells	CD45+CD66b-
	CD45RA+CD45RO-		CD3+CD19-
07 CD8 T cells, central memory (CM)	CD45+CD66b-		CD20-CD45+
	CD3+CD19-		CD14-CD45+
	CD20-CD45+		gdTCR-CD3+
	CD14-CD45+		CD4+CD8-
	gdTCR-CD3+		
	CD8+CD4-		
	CCR7+CD8+		
	CD45RA-CD45RO+		

Table 1. Gates used to distinguish immune cell populations stained by the Human Immune Monitoring Panel Kit.

Immune Populations	Gates	Immune Populations	Gates
12 CD4 T cells, naive	CD45+CD66b-	**16** CD4 T cells, activated	CD45+CD66b-
	CD3+CD19-		CD3+CD19-
	CD20-CD45+		CD20-CD45+
	CD14-CD45+		CD14-CD45+
	gdTCR-CD3+		gdTCR-CD3+
	CD4+CD8-		CD4+CD8-
	CCR7+CD4+		HLADR+CD38+
	CD45RA+CD45RO-	**17** Total CD4+ Treg	CD45+CD66b-
13 CD4 T cells, central memory (CM)	CD45+CD66b-		CD3+CD19-
	CD3+CD19-		CD20-CD45+
	CD20-CD45+		CD14-CD45+
	CD14-CD45+		gdTCR-CD3+
	gdTCR-CD3+		CD4+CD8-
	CD4+CD8-		CD25+CD127-
	CCR7+CD4+	**18** Total CCR4+ CD4+ Treg	CD45+CD66b-
	CD45RA-CD45RO+		CD3+CD19-
14 CD4 T cells, effector memory (EM)	CD45+CD66b-		CD20-CD45+
	CD3+CD19-		CD14-CD45+
	CD20-CD45+		gdTCR-CD3+
	CD14-CD45+		CD4+CD8-
	gdTCR-CD3+		CD25+CD127-
	CD4+CD8-		CCR4+CD4+
	CCR7-CD4+	**19** CCR4+ CD4 Treg naive	CD45+CD66b-
	CD45RA-CD45RO+		CD3+CD19-
15 CD4 T cells, terminal effector (TE)	CD45+CD66b-		CD20-CD45+
	CD3+CD19-		CD14-CD45+
	CD20-CD45+		gdTCR-CD3+
	CD14-CD45+		CD4+CD8-
	gdTCR-CD3+		CD25+CD127-
	CD4+CD8-		CCR4+CD4+
	CCR7-CD4+		CD45RA+CD45RO-
	CD45RA+CD45RO-		

Table 1. Gates used to distinguish immune cell populations stained by the Human Immune Monitoring Panel Kit.

Immune Populations	Gates	Immune Populations	Gates
20 Total CCR4+ CD4+ Treg activated	CD45+CD66b-	**24** Th17	CD45+CD66b-
	CD14-CD45+		CD14-CD45+
	CD20-CD45+		CD20-CD45+
	CD3+CD19-		CD3+CD19-
	gdTCR-CD3+		gdTCR-CD3+
	CD4+CD8-		CD4+CD8-
	CD25+CD127-		CXCR5-CD45+
	CCR4+CD4+		CCR6+CXCR3-
	HLADR+CD45+	**25** B cells	CD45+CD66b-
21 CCR4+ CD4 Treg memory	CD45+CD66b-		CD19+CD3-
	CD3+CD19-		CD14-CD45+
	CD20-CD45+		CD16-CD161-
	CD14-CD45+	**26** Naive B cells	CD45+CD66b-
	gdTCR-CD3+		CD19+CD3-
	CD4+CD8-		CD14-CD45+
	CD25+CD127-		CD16-CD161-
	CCR4+CD4+		CD20+CD45+
	CD45RA-CD45RO+		IgD+CD27-
22 Th1	CD45+CD66b-	**27** B cells, IgD- memory	CD45+CD66b-
	CD14-CD45+		CD19+CD3-
	CD20-CD45+		CD14-CD45+
	CD3+CD19-		CD16-CD161-
	gdTCR-CD3+		CD20+CD45+
	CD4+CD8-		IgD-CD27+
	CXCR5-CD45+	**28** B cells, IgD+ memory	CD45+CD66b-
	CXCR3+CCR6-		CD19+CD3-
23 Th2	CD45+CD66b-		CD14-CD45+
	CD14-CD45+		CD16-CD161-
	CD20-CD45+		CD20+CD45+
	CD3+CD19-		IgD+CD27+
	gdTCR-CD3+	**29** Transitional B cells	CD45+CD66b-
	CD4+CD8-		CD19+CD3-
	CXCR5-CD45+		CD14-CD45+
	CCR6-CXCR3-		CD16-CD161-
			CD20+CD45+
			CD24+CD38+

Table 1. Gates used to distinguish immune cell populations stained by the Human Immune Monitoring Panel Kit.

Immune Populations	Gates	Immune Populations	Gates
30 Plasmablasts	CD45+CD66b-	**36** CD11c+CD123- mDC	CD45+CD66b-
	CD19+CD3-		CD19-CD3-
	CD14-CD45+		CD20-CD45+
	CD16-CD161-		CD14-CD45+
	CD20-CD45+		HLADR+CD56-
	CD27+CD38+		CD11c+CD123-
31 Total monocytes	CD45+CD66b-		CD16-CD45+
	CD14+CD45+	**37** NK cells	CD45+CD66b-
	CD19-CD3-		CD19-CD3-
	CD20-CD45+		CD14-CD45+
32 Non-classical monocytes	CD45+CD66b-		CD20-CD45+
	CD14+CD45+		CD56+CD123-
	CD19-CD3-		CD161+CD56+
	CD20-CD45+	**38** CD16- NK cells	CD45+CD66b-
	CD16+CD14+		CD19-CD3-
33 Classical monocytes	CD45+CD66b-		CD14-CD45+
	CD14+CD45+		CD20-CD45+
	CD19-CD3-		CD56+CD123-
	CD20-CD45+		CD161+CD56+
	CD16-CD14+		CD16-CD161+
34 Total DCs	CD45+CD66b-	**39** CD16+ NK cells	CD45+CD66b-
	CD19-CD3-		CD19-CD3-
	CD20-CD45+		CD14-CD45+
	CD14-CD45+		CD20-CD45+
	HLADR+CD56-		CD56+CD123-
35 CD11c- CD123+ pDC	CD45+CD66b-		CD161+CD56+
	CD19-CD3-		CD16+CD161+
	CD20-CD45+	**40** Gamma-delta T cells	CD45+CD66b-
	CD14-CD45+		CD3+CD19-
	HLADR+CD56-		CD20-CD45+
	CD11c-CD123+		CD14-CD45+
	CD16-CD45+		gdTCR+CD3+

Table 2. Gates used to distinguish intermediate cell populations stained by the Human Immune Monitoring Panel Kit.

Intermediate Populations	Gates	Intermediate Populations	Gates
CD14+CD45+	CD45+CD66b-	CCR7-CD4+	CD45+CD66b-
	CD14+CD45+		CD3+CD19-
CD19+CD3-	CD45+CD66b-		CD20-CD45+
	CD19+CD3-		CD14-CD45+
CD3+CD19-	CD45+CD66b-		gdTCR-CD3+
	CD3+CD19-		CD4+CD8-
Non-T/B, non-mono	CD45+CD66b-		CCR7-CD4+
	CD19-CD3-	CCR7+CD8+	CD45+CD66b-
	CD14-CD45+		CD3+CD19-
	CD20-CD45+		CD20-CD45+
Th cells	CD45+CD66b-		CD14-CD45+
	CD14-CD45+		gdTCR-CD3+
	CD20-CD45+		CD8+CD4-
	CD3+CD19-		CCR7+CD8+
	gdTCR-CD3+	CCR7-CD8+	CD45+CD66b-
	CD4+CD8-		CD3+CD19-
	CXCR5-CD45+		CD20-CD45+
CCR7+CD4+	CD45+CD66b-		CD14-CD45+
	CD3+CD19-		gdTCR-CD3+
	CD20-CD45+		CD8+CD4-
	CD14-CD45+		CCR7-CD8+
	gdTCR-CD3+		
	CD4+CD8-		
	CCR7+CD4+		

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