Analyzing Protein Phosphorylation Pathways in Heterogeneous Samples

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BD Biosciences, San Diego, CA

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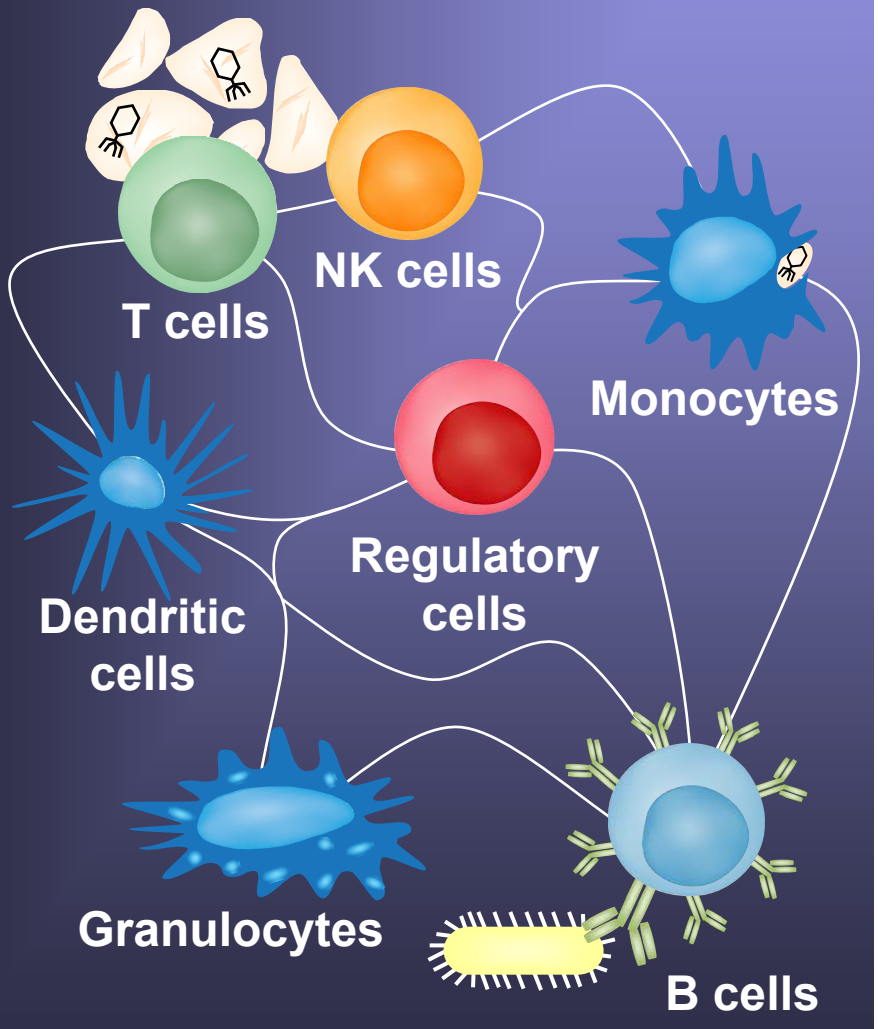
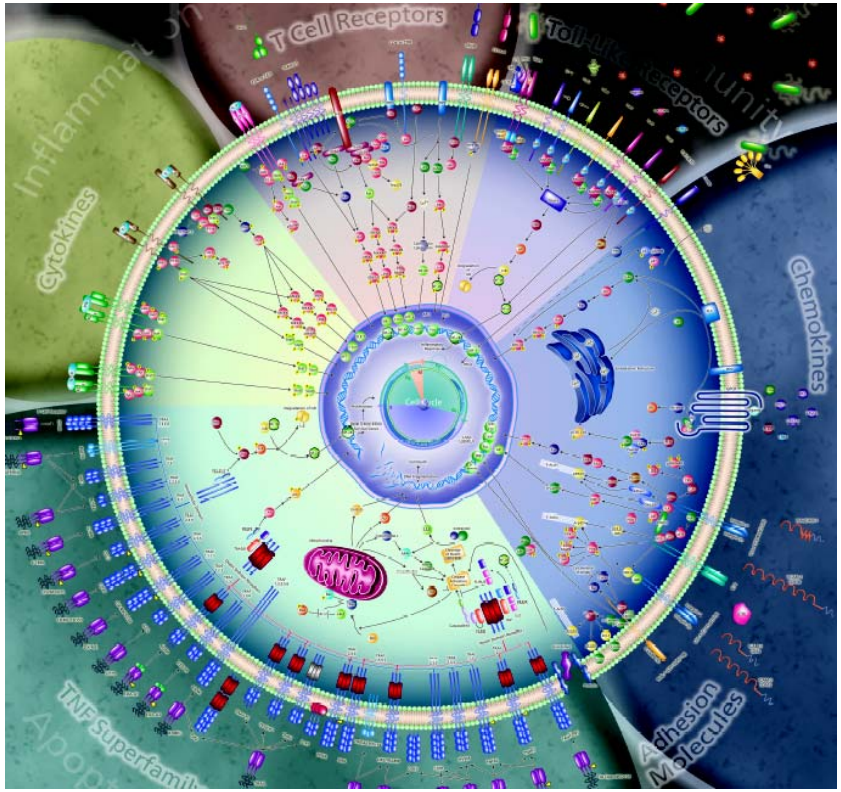
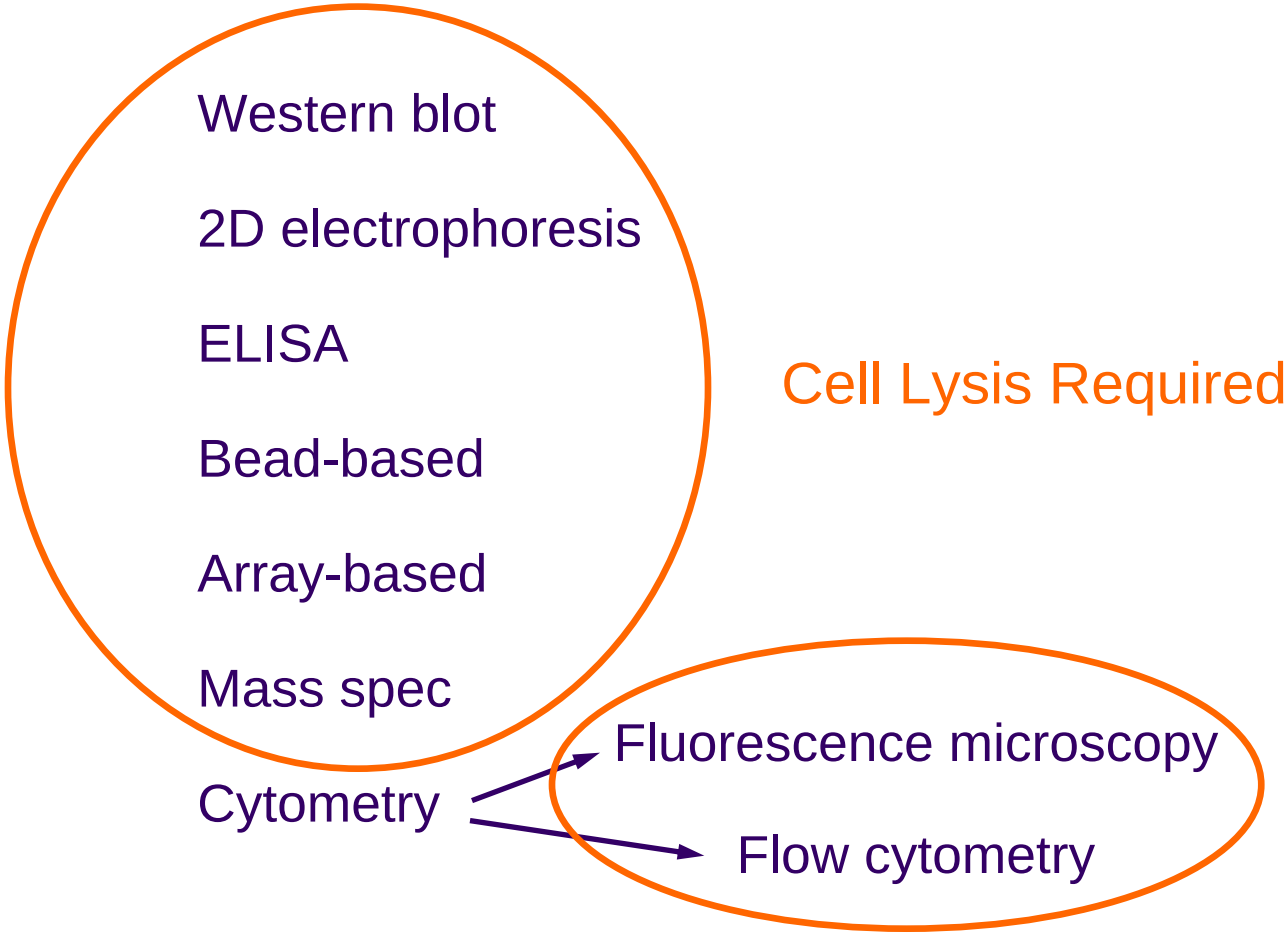
A Critical Role for Cell Signaling in Communication within the Immune System





BD

Many Tools Are Available Today for Studying Signal Transduction



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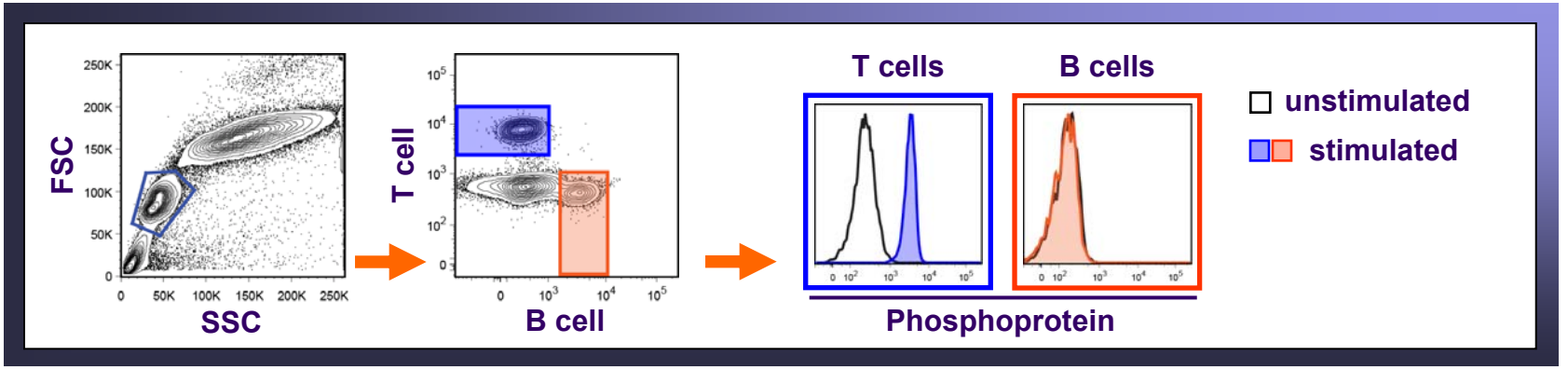
Many Tools Are Available Today for Studying Signal Transduction

Fluorescence Microscopy

Flow Cytometry



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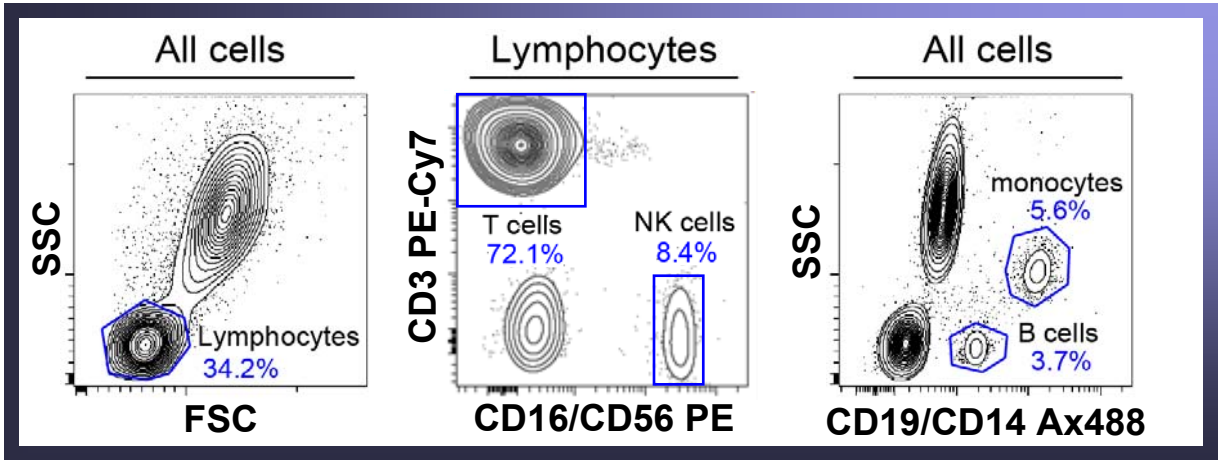
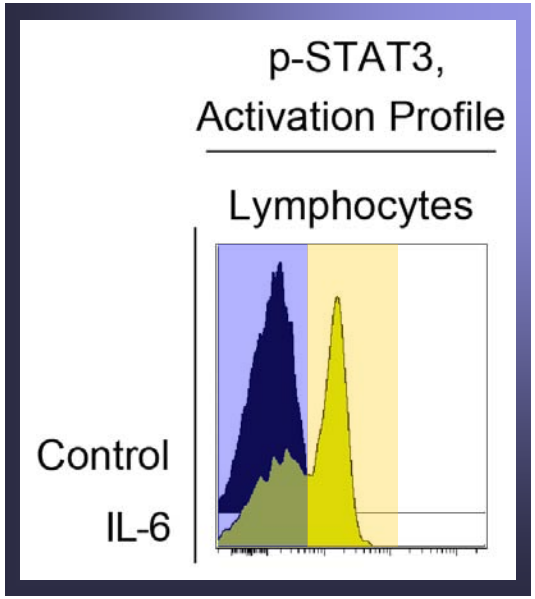
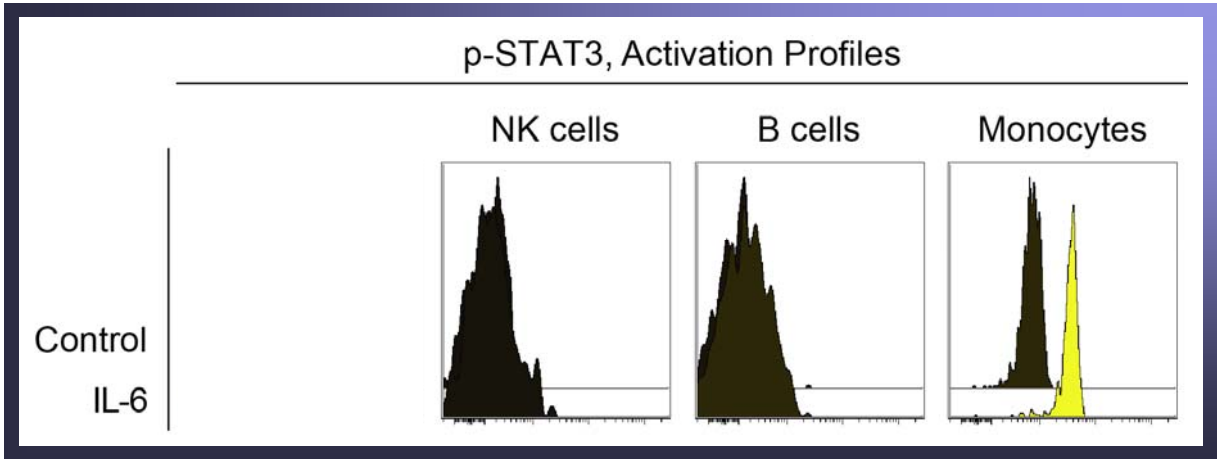
Monocyte / NK Cell Activation Kit: Signaling Responses in Human Leucocyte Subsets

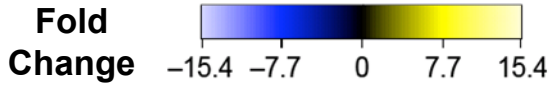
Human whole blood stimulated with the pro-inflammatory cytokine IL-6







Which lymphocytes are responding to IL-6?



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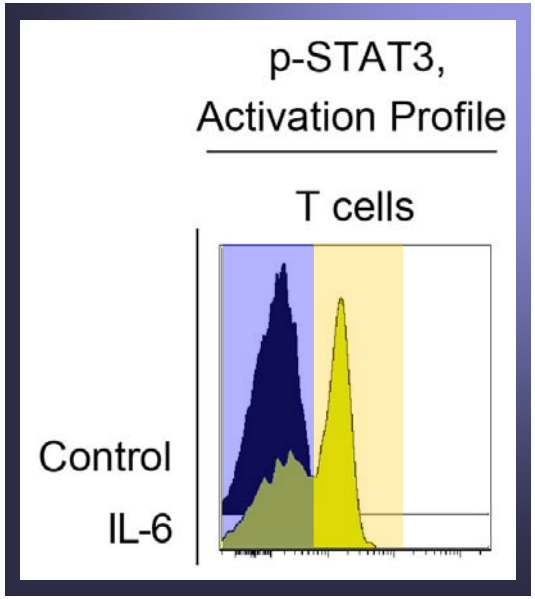
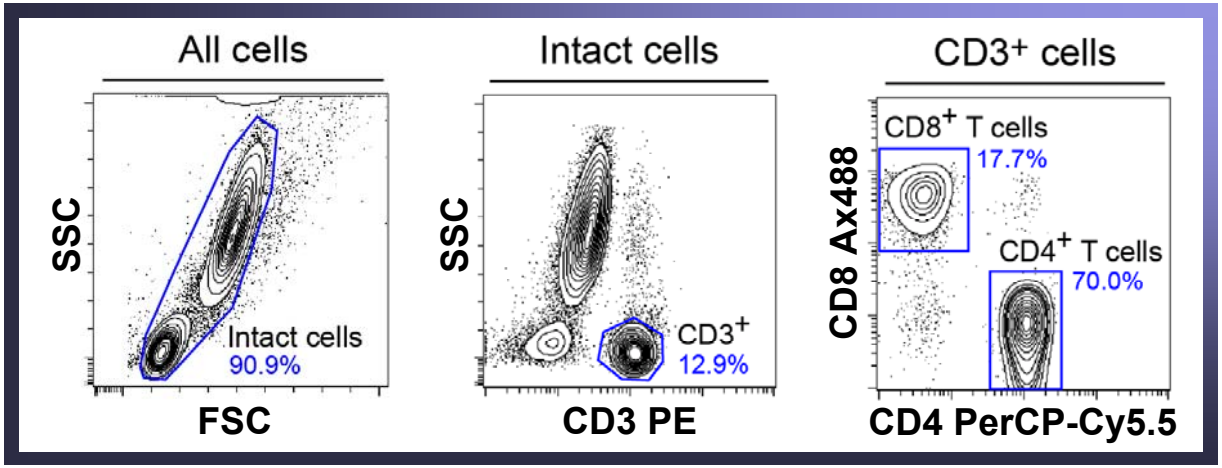
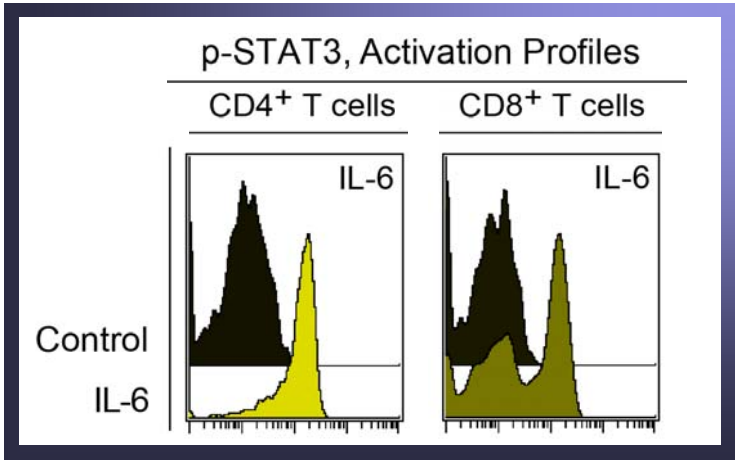
T Cell Activation Kit: Measuring Signaling Responses in CD4 and CD8 T Cells

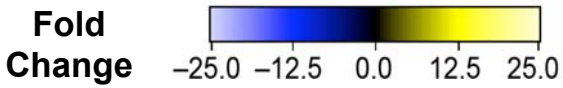
Human whole blood stimulated with the pro-inflammatory cytokine IL-6



Which T cells are responding to IL-6?







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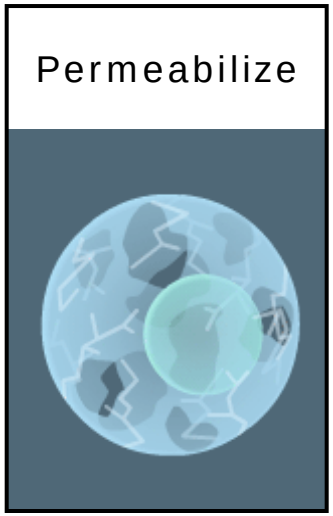
Standard Protocol for Analyzing Protein Phosphorylation by Flow Cytometry

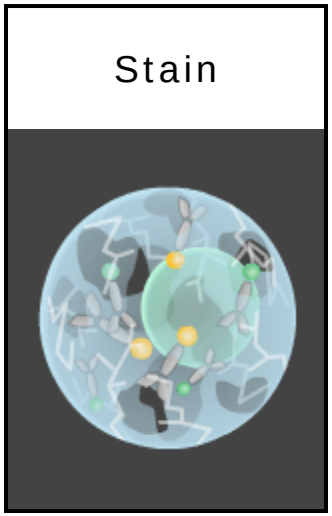
Step 1

Stimulate cells (optional) and fix to preserve phosphorylation states



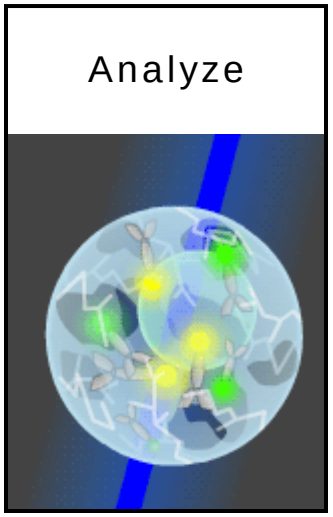
Step 2

Permeabilize cells to allow antibody access to cytoplasm and nucleus



Step 3

Stain cells with fluorescently conjugated antibodies against phosphoepitopes, surface markers, and other targets of interest



Step 4

Analyze cells on a properly set up flow cytometer

If working with whole blood, spleen, or other erythrocyte-containing samples, RBC lysis can be performed during fixation using BD Phosflow™ Lyse/Fix Buffer

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Elements Required for Successful Analysis of Cell Signaling at the Single-Cell Level

- Phospho-specific antibodies validated for flow cytometry
  - Specific
  - High S/N
  - Consistent
- Optimized buffer systems for fixation and permeabilization
  - Different buffer options for different sample types and phosphoepitopes
- Strategy for identification of cell populations of interest
  - Compatibility with fixation and permeabilization buffers
  - Optimization of staining conditions
- Viable and healthy samples
  - Ex vivo stimulation to trigger phospho-signaling networks
  - Detection of altered basal phosphorylation states
- Robust results
  - Careful panel design and instrument setup
  - Consistent staining
- Data analysis tools

bdbiosciences.com/phosflow

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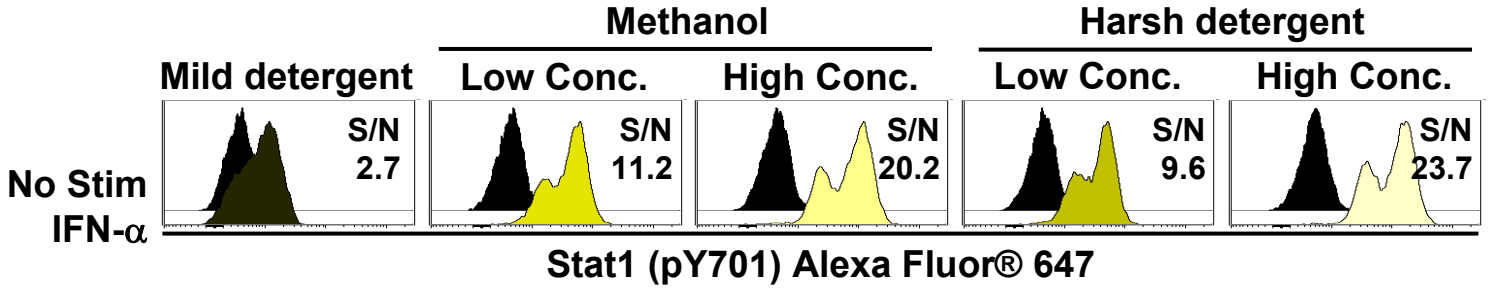
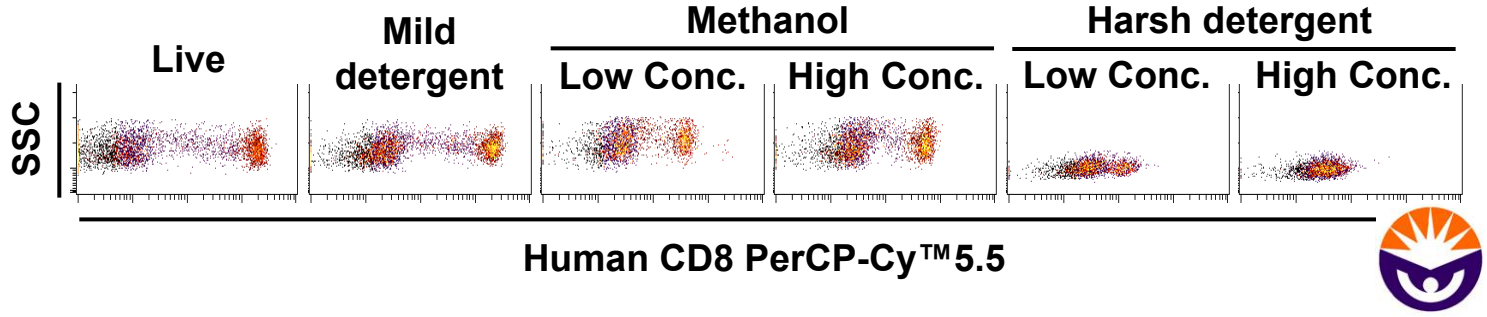
Fixation and Permeabilization: Selecting the Best Protocol

- **Multiple fixation and permeabilization buffers are available**
  - **Appropriate buffer choice is critical for successful detection of phosphoproteins, surface markers, and other proteins of interest (eg, transcription factors, cell cycle or apoptosis proteins, etc.)**
- **Harsh, denaturing conditions favor detection of some phosphoproteins**



- **Fixation and permeabilization can adversely affect the detection of some surface markers, with harsh buffers causing more severe effects**



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Selecting Fixation and Permeabilization Buffers: Fixation

- **Formaldehyde-based fixation prior to permeabilization provides optimal phosphoprotein detection and FSC/SSC resolution**
  - **Formaldehyde stability and concentration are critical**
  - **Use a source recommended in established protocols**

- **Sample type determines fixative choice**

Sample Type	Fixative
Whole blood, spleen, or other erythrocyte-containing samples	BD Phosflow Lyse/Fix Buffer
PBMCs, cell lines, etc.	BD Cytofix™ Fixation Buffer

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Selecting Fixation and Permeabilization Buffers: Permeabilization

BD Phosflow Perm Buffers			
Perm/Wash Buffer I	**Perm Buffer II**	**Perm Buffer III**	**Perm Buffer IV**
• Mild detergent (saponin) method • Easiest on cell-surface markers • Adequate for detection of nuclear and cytoplasmic proteins but suboptimal for Stat pY detection	• Mild alcohol method (low conc. methanol) • Few cell-surface markers lost • Good for intracellular staining	• Harsh alcohol method (high conc. methanol) • Some cell-surface markers lost • Best for many intracellular markers • Most similar to Nolan lab method • Recommended starting protocol	• Harsh detergent method • Some cell-surface markers lost • Best for Stat pY and certain other intracellular markers • May result in greater cell loss than other buffers

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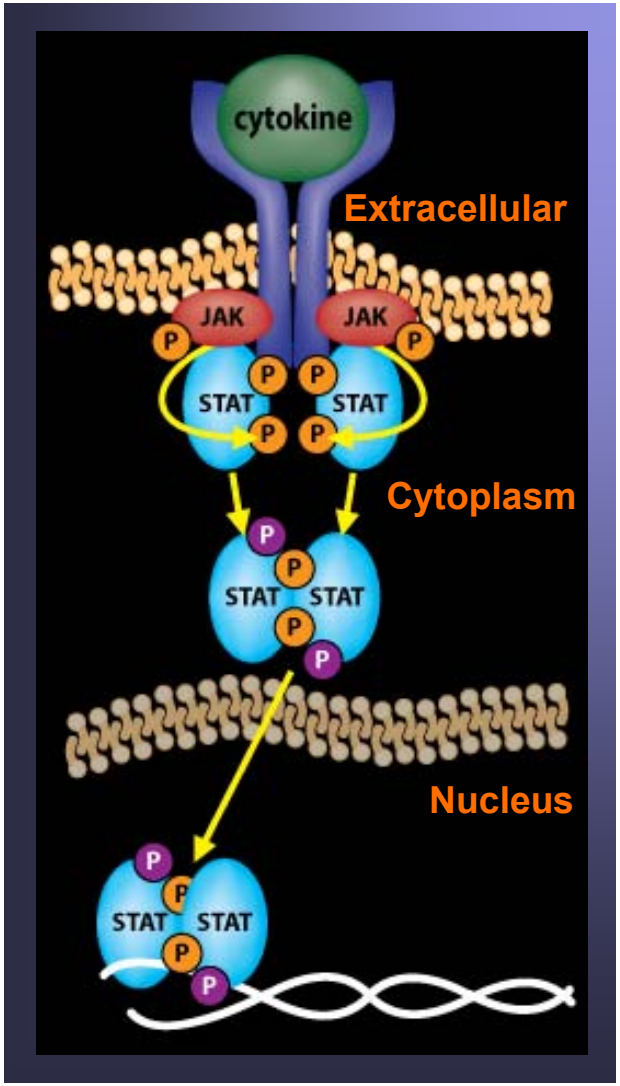
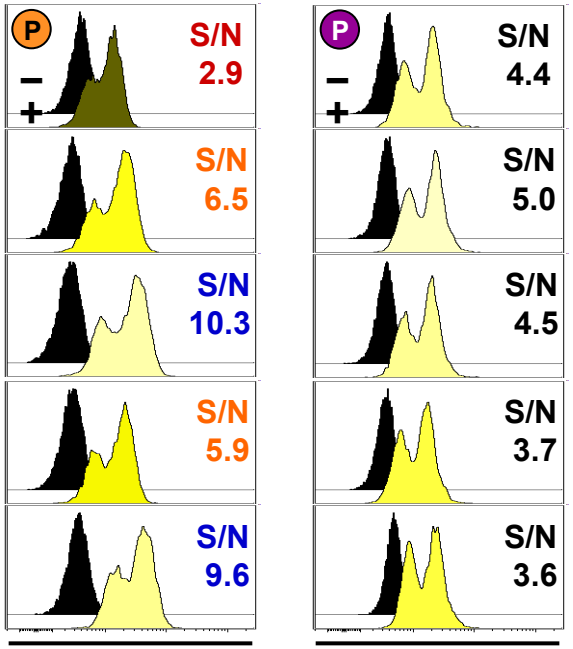
Selecting Fixation and Permeabilization Buffers: Permeabilization



- **Phosphoprotein detection requirements:**
  - Access to cytoplasmic and/or nuclear proteins
  - Some phosphoepitopes favor harsh, denaturing permeabilization buffers



Mild Detergent (Saponin)
Perm/Wash Buffer I

Methanol – Low Conc.
Perm Buffer II

Methanol – High Conc.
Perm Buffer III

Harsh Detergent – Low Conc.
Perm Buffer IV (0.5X)

Harsh Detergent – High Conc.
Perm Buffer IV (1X)

Human PBMCs activated with IFN-α (pY701) or PMA (pS727) for 15 min and fixed with BD Cytofix

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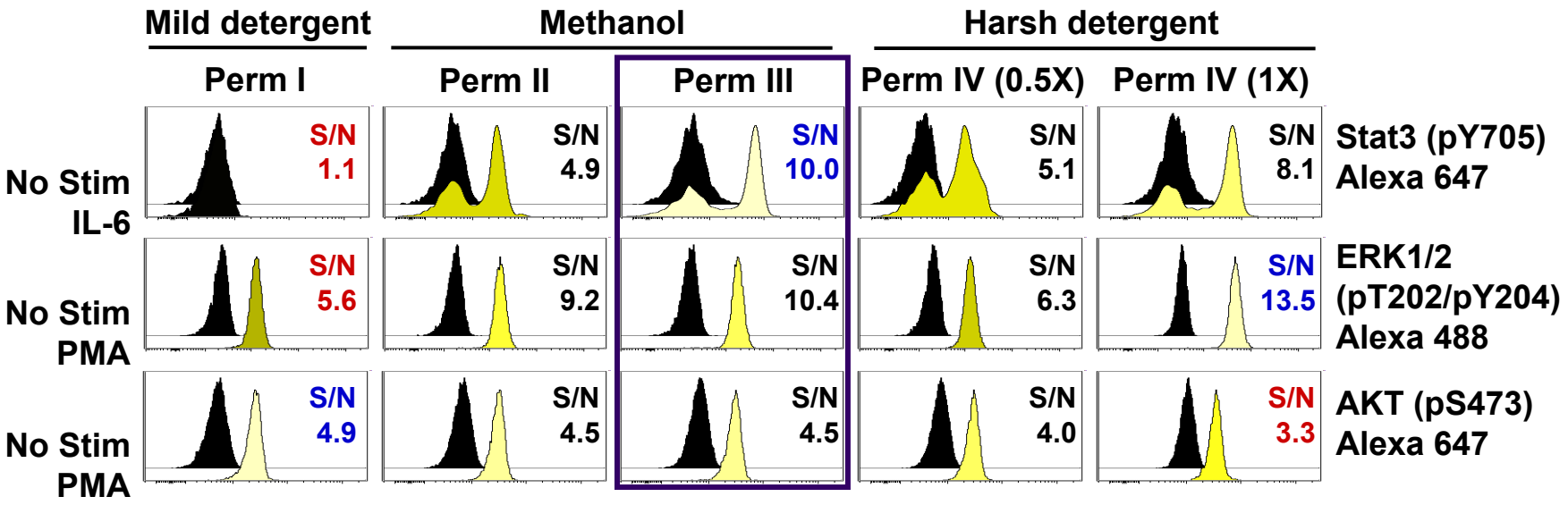
Permeabilization Buffer Selection Impacts Phosphoprotein Staining



Human whole blood (Stat3) or PBMCs (ERK and AKT) activated with IL-6 or PMA for 15 min and fixed with BD Phosflow Lyse/Fix Buffer (whole blood) or BD Cytofix Buffer (PBMCs)

- **Harsh permeabilization buffers provide superior staining of many, but not all, phosphoepitopes**
  - **High-concentration methanol (Perm III) and harsh detergent buffer (Perm IV)**
- **Vast majority of BD Phosflow™ antibodies work with Perm Buffer III, although some yield superior staining with other buffers**
  (eg, CREB pS133 and IκBα antibodies work best with Perm Buffer II)

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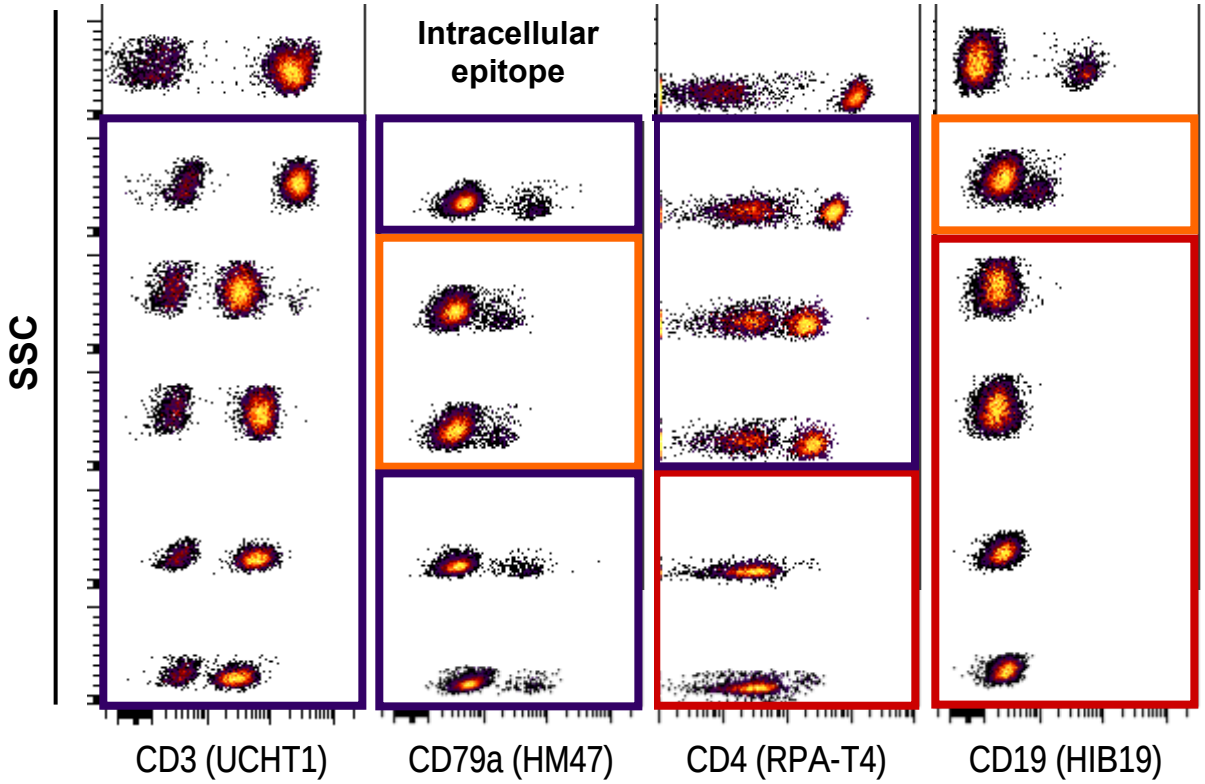
Permeabilization Buffer Selection Impacts Surface Marker Resolution

- Perm/Wash Buffer I usually has the mildest effects on surface marker staining
- Different effects of methanol-based (Perm II and III) vs harsh-detergent (Perm IV) buffers on different epitopes



Live Stain
BD FACS™ Lysing Solution post-stain

Mild Detergent (Saponin)
Perm/Wash Buffer I

Methanol – Low Conc.
Perm Buffer II

Methanol – High Conc.
Perm Buffer III

Harsh Detergent – Low Conc.
Perm Buffer IV (0.5X)

Harsh Detergent – High Conc.
Perm Buffer IV (1X)

Human whole blood fixed with BD Phosflow Lyse/Fix Buffer, permeabilized, and stained with PerCP-Cy™5.5–conjugated antibodies

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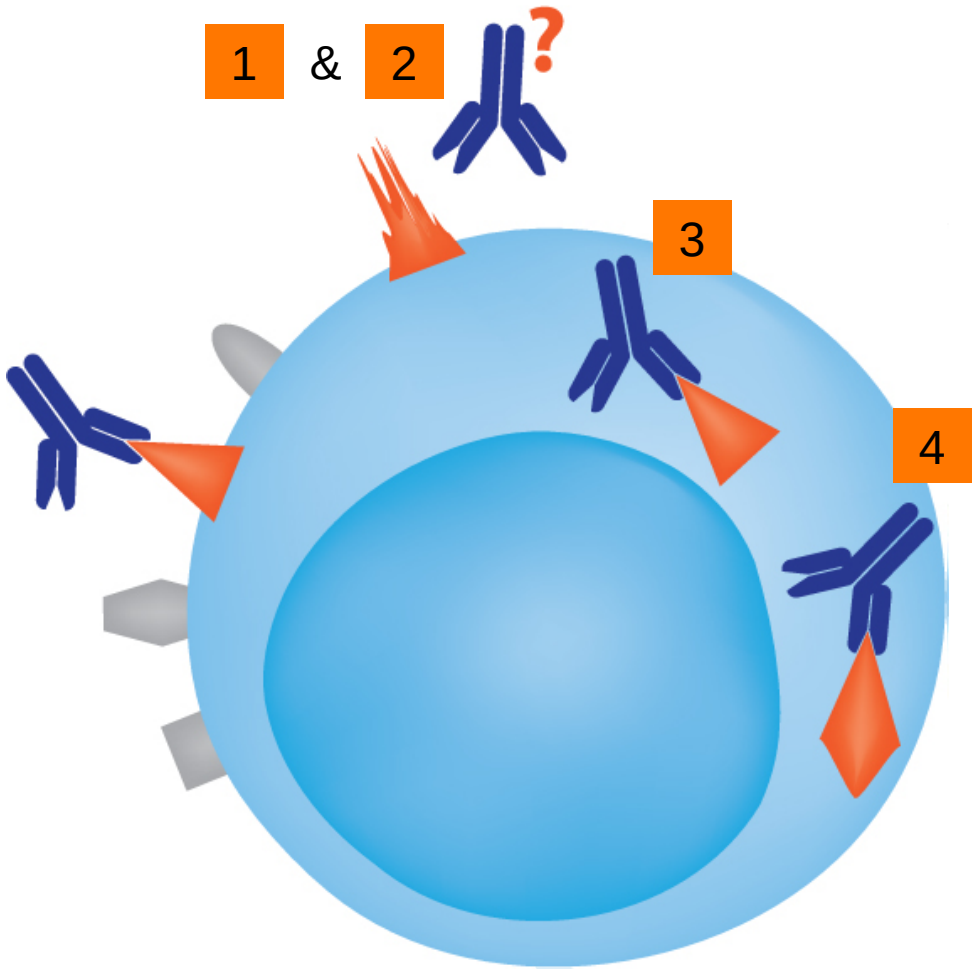
Why is Resolution of Surface Marker Stains Reduced in Permeabilized Cells?



1 Fixation covalently modifies surface marker epitopes, preventing antibody binding

2 Harsh permeabilization buffers denature epitopes, preventing antibody binding

3 Permeabilization allows antibodies access to intracellular stores of antigen

4 Permeabilization opens up access to epitopes that were inaccessible during antibody screening, increasing nonspecific background

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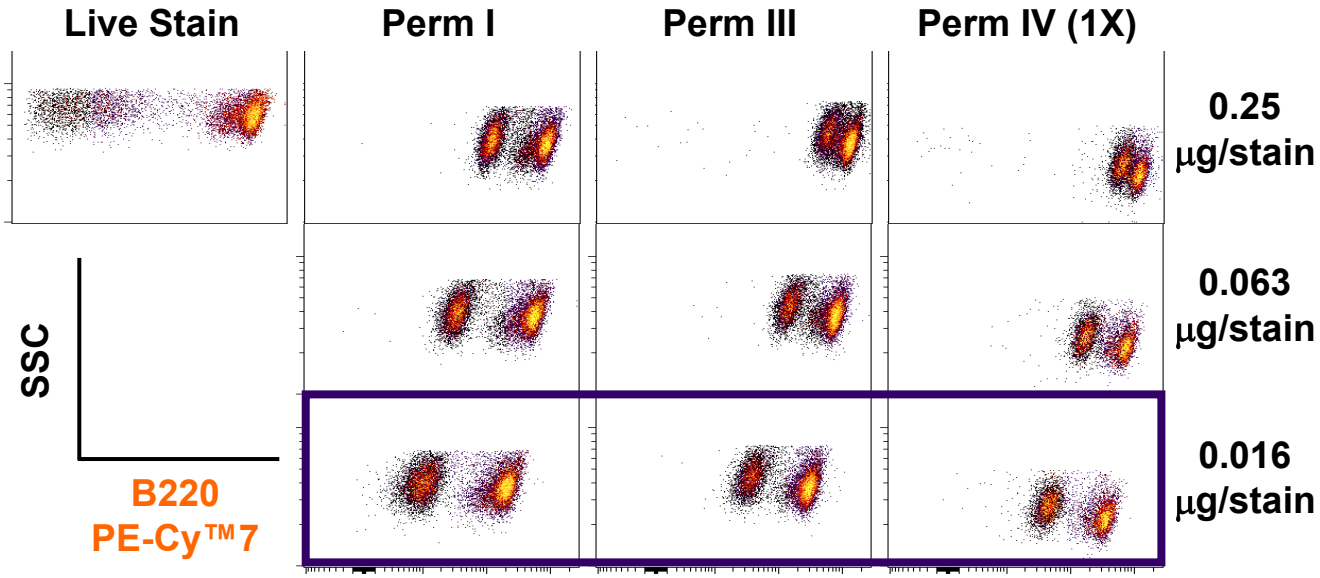
Antibody Titration Can Improve Surface Marker Resolution

Post-perm staining of anti-mouse B220 antibody is optimal at a concentration far below that used for live cell stains



BALB/c mouse spleen cells fixed with BD Phosflow Lyse/Fix Buffer, permeabilized, and stained

4 **Permeabilization opens up access to epitopes that were inaccessible during antibody screening, increasing nonspecific background**

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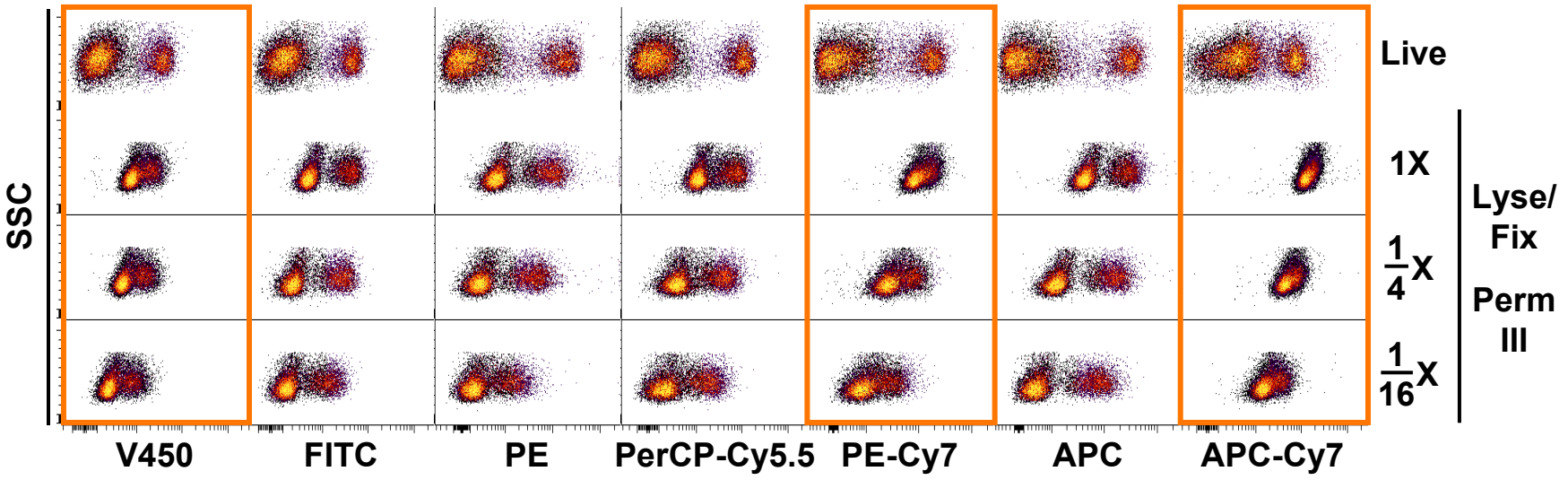
Post-Perm Staining Success May Differ for Different Fluorescent Conjugates

Very high background staining and/or low signal can prevent some fluorophore conjugates of an antibody from working well in post-permeabilization stains



BALB/c mouse spleen cells fixed with BD Phosflow Lyse/Fix Buffer, permeabilized, and stained with various fluorophore conjugates of anti-TCRβ antibody (H57-597)

4 **Permeabilization opens up access to epitopes that were inaccessible during antibody screening, increasing nonspecific background**

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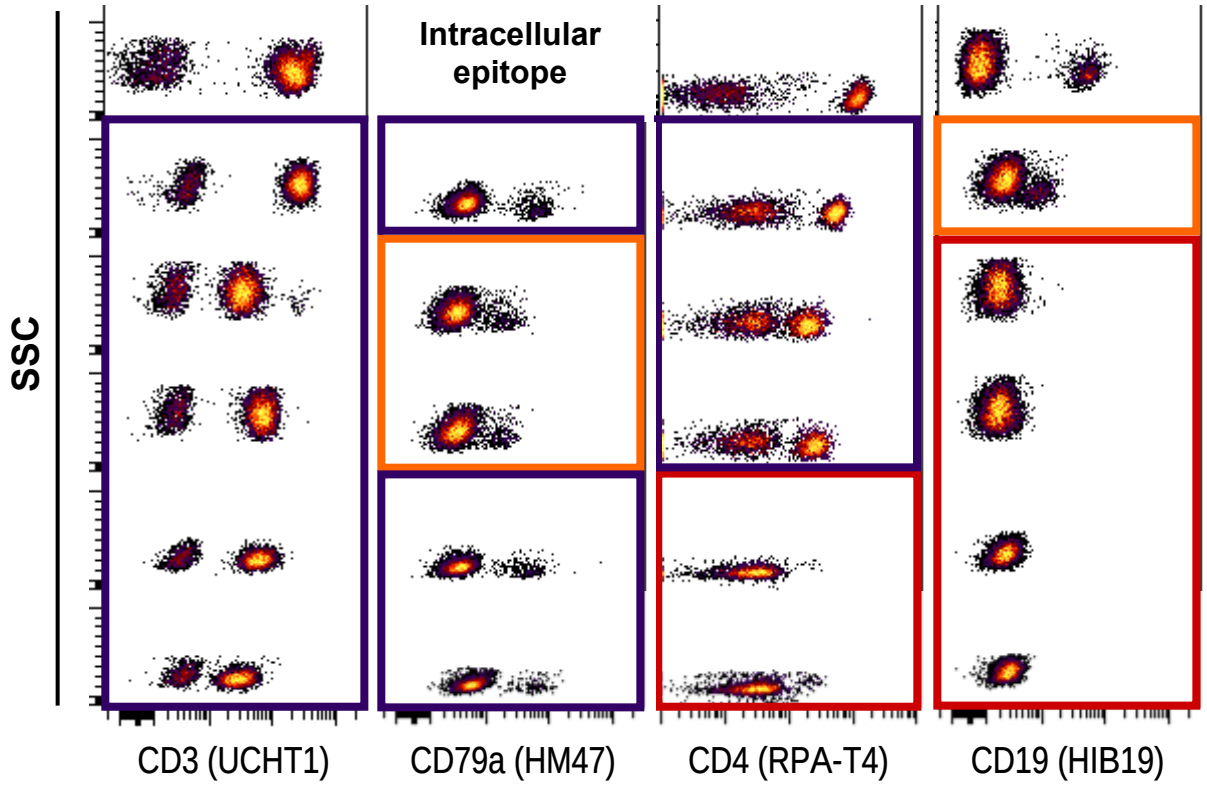
Permeabilization Buffer Selection Impacts Surface Marker Resolution



Live Stain
BD FACS Lysing Solution post-stain

Mild Detergent (Saponin)
Perm/Wash Buffer I

Methanol – Low Conc.
Perm Buffer II

Methanol – High Conc.
Perm Buffer III

Harsh Detergent – Low Conc.
Perm Buffer IV (0.5X)

Harsh Detergent – High Conc.
Perm Buffer IV (1X)

Human whole blood fixed with BD Phosflow Lyse/Fix Buffer, permeabilized, and stained with PerCP-Cy5.5–conjugated antibodies

2 **Harsh permeabilization buffers denature epitopes, preventing antibody binding**

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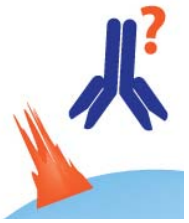
Differential Effects of Fix/Perm on Epitopes: Antibody Clone Choice is Important

Antibodies to Human Cell-Surface Markers Tested for BD Phosflow Protocols

Specificity	Clone	Fluorochrome	Protocol I *Detergent method*	Protocol II *Mild alcohol method*	Protocol III *Harsh alcohol method*	Protocol IV *Detergent method*
Human CD3	SK7	APC	+	+	+	+
		APC-Cy™7	+	+	–	+
		FITC	+	+	+	+
		PE	+	+	+	+
		PE-Cy™7	+	+	+	+
		PerCP	+	+	+/–	–
		PerCP-Cy™5.5	+	+	+	+
	UCHT1	Alexa Fluor® 488	+	+	+	+
		Alexa Fluor® 647	+	+	+	+
		Alexa Fluor® 700		+	+/–	–
		APC	+	+	+	+
		FITC	+	+	+	+
		BD Horizon™ V450			+	+
		Pacific Blue™		+	+	+
		PE	+	+	+	+
		PE-Cy™5	+	+	+	+
		PE-Cy7	+	+	+	+
	HIT3a	APC	+	–	–	+/–
		FITC		–	–	
		PE	+	–	–	–
		PE-Cy5	+		–	–
	SP34	PE-Cy7	+		–	+/–
		PerCP	+	+	+	–



1 **Fixation covalently modifies surface marker epitopes, preventing antibody binding**

2 **Harsh permeabilization buffers denature epitopes, preventing antibody binding**

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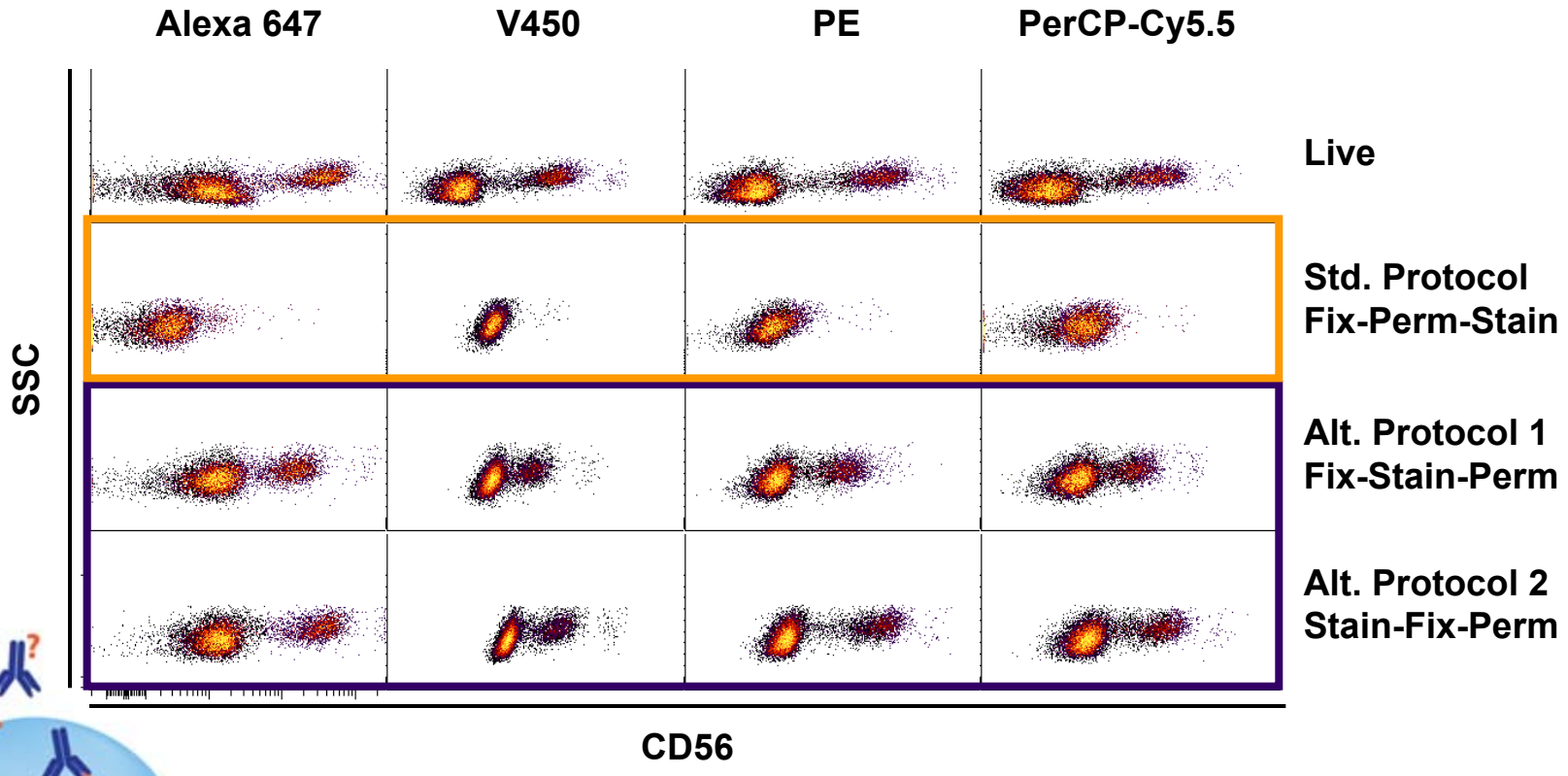
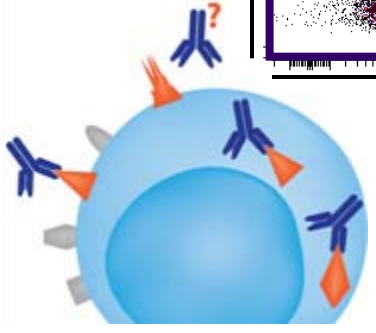
Alternative Staining Protocols Can Improve Surface Marker Resolution

Resolution of some surface markers can be improved by staining before permeabilization (Alt. Protocol 1) or before fixation (Alt. Protocol 2)





Human whole blood fixed with BD Phosflow Lyse/Fix Buffer and permeabilized with BD Phosflow Perm/Wash Buffer I

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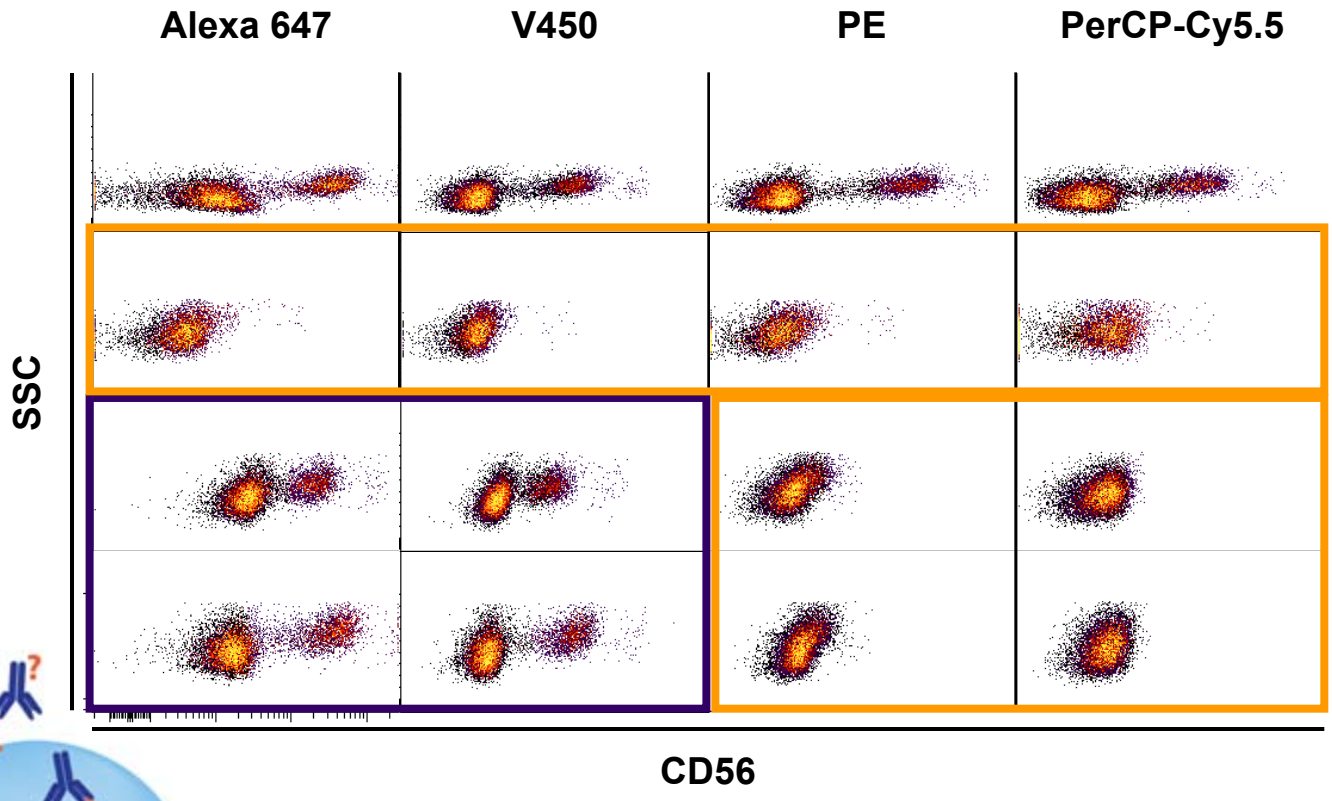
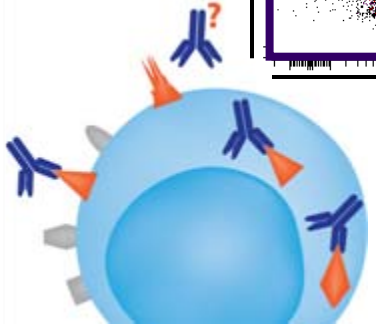
Fluorophore Choice for Alternative Staining Protocols: Perm Buffer III

Protein fluorophores are destroyed by exposure to methanol-containing permeabilization buffer





Human whole blood fixed with BD Phosflow Lyse/Fix Buffer and permeabilized with BD Phosflow Perm Buffer III

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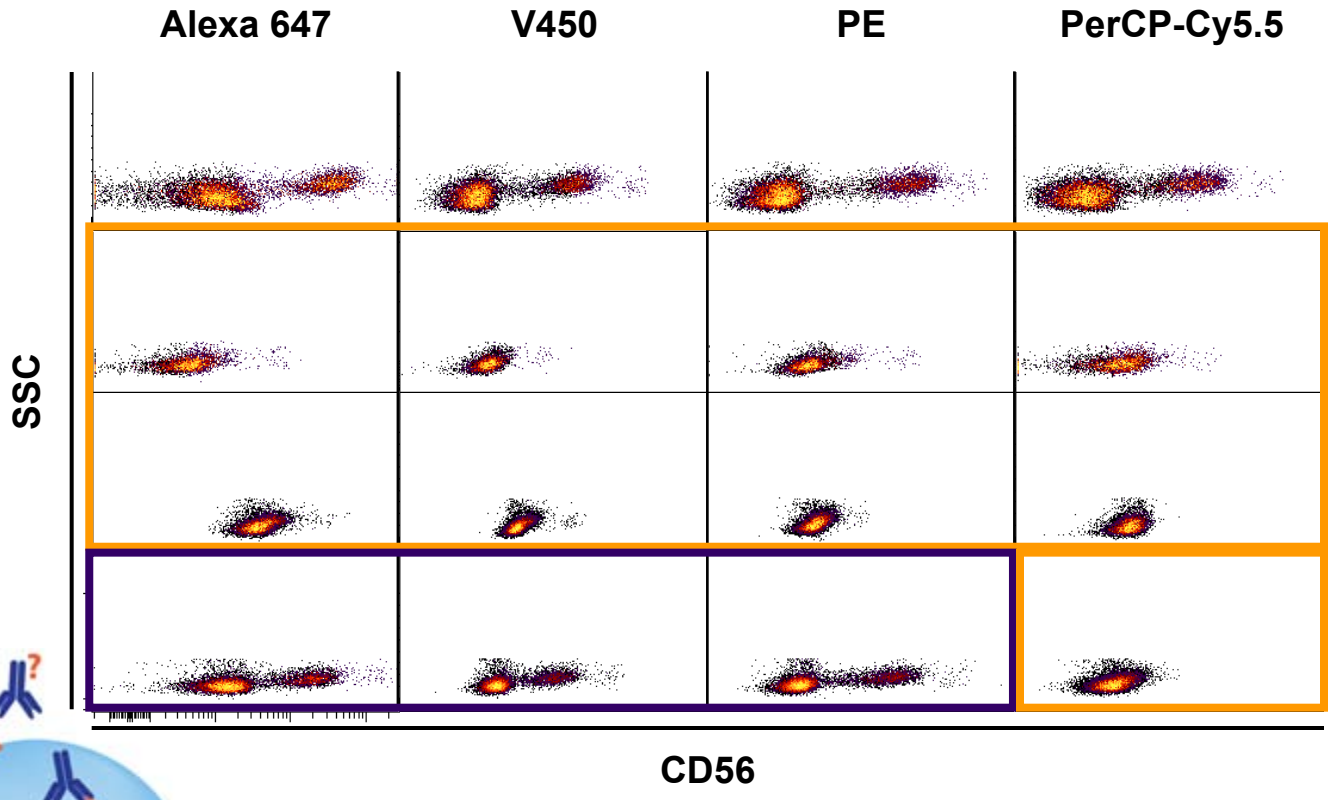
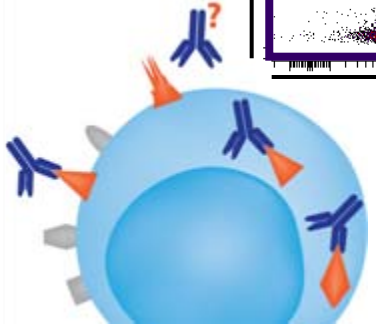
Fluorophore Choice for Alternative Staining Protocols: Perm Buffer IV

Many fluorophores are damaged by the harsh detergent-containing BD Phosflow Perm Buffer IV, but fixation stabilizes some stains





Human whole blood fixed with BD Phosflow Lyse/Fix Buffer and permeabilized with BD Phosflow Perm Buffer IV (1X)

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BD FACSelect™ Buffer Compatibility Resource

Goal:

- To create a resource to facilitate the design of multiparameter staining panels for simultaneous analysis of intracellular and surface marker proteins

Approach:

- Generate data for key intracellular and surface marker specificities using available fluorochromes and various fixation and permeabilization protocols

Variables:

- Sample types: Human whole blood & PBMCs, murine splenocytes, & bone marrow
- Surface and intracellular specificities in all available fluorochromes
- Fixatives: BD Phosflow Lyse/Fix Buffer (human whole blood, mouse cells) or BD Cytofix Fixation Buffer (human PBMCs, cell lines)
- Permeabilization buffers: BD Phosflow Perm Buffers I–IV (IV at 1X and 0.5X conc.)
- Antibody concentrations: Three-point titrations of all surface marker antibodies
- Surface marker staining protocols: Standard Protocol (Fix-Perm-Stain), Alternative Protocol 1 (Fix-Stain-Perm), and Alternative Protocol 2 (Stain-Fix-Perm)

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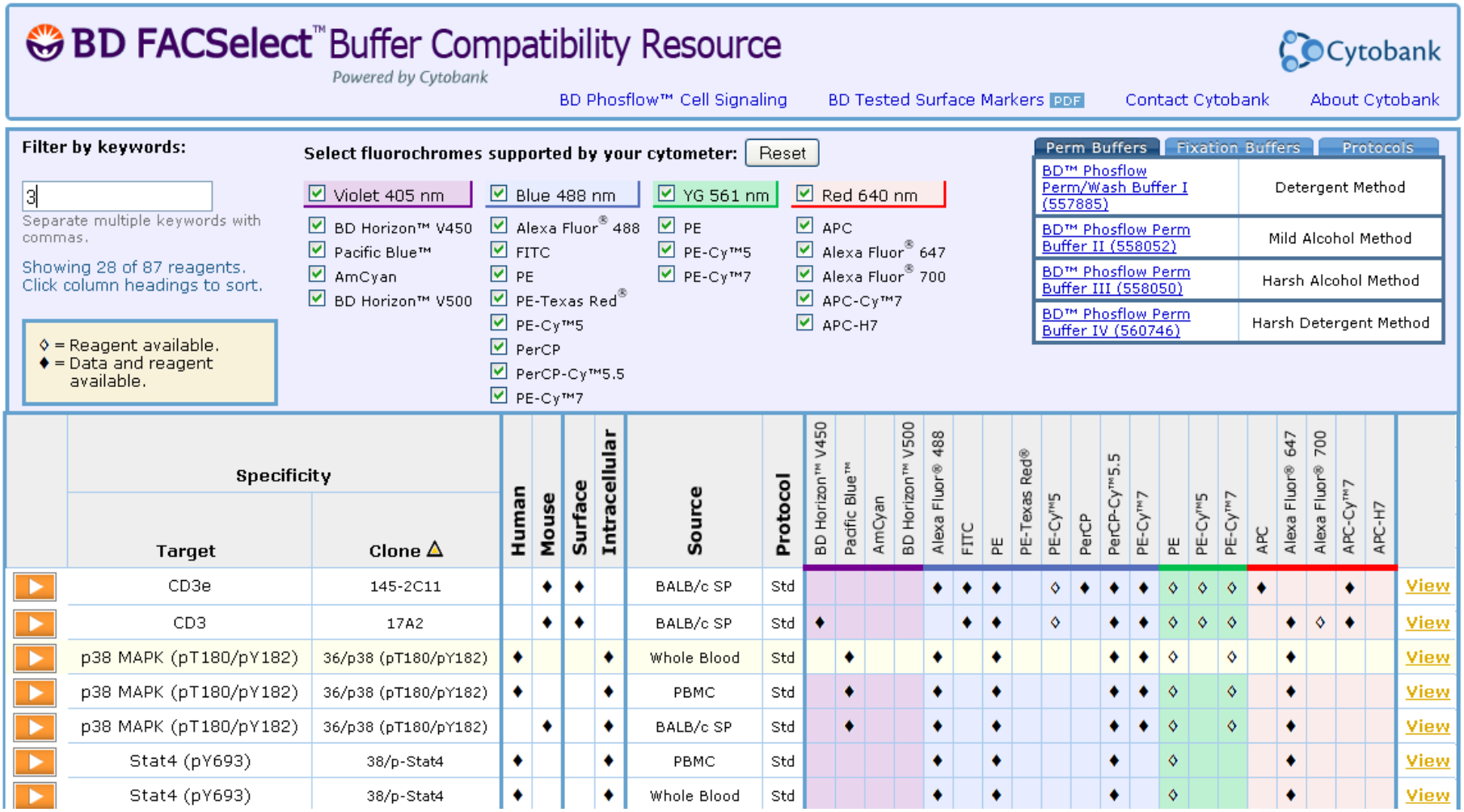
BD FACSelect™ Buffer Compatibility Resource



BD FACSelect™ Buffer Compatibility Resource

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Filter by keywords:

3

Separate multiple keywords with commas.

Showing 28 of 87 reagents.
Click column headings to sort.

◊ = Reagent available.
♦ = Data and reagent available.

Select fluorochromes supported by your cytometer: Reset

- Violet 405 nm: BD Horizon™ V450, Pacific Blue™, AmCyan, BD Horizon™ V500
- Blue 488 nm: Alexa Fluor® 488, FITC, PE, PE-Texas Red®, PE-Cy™5, PerCP, PerCP-Cy™5.5, PE-Cy™7
- YG 561 nm: PE, PE-Cy™5, PE-Cy™7
- Red 640 nm: APC, Alexa Fluor® 647, Alexa Fluor® 700, APC-Cy™7, APC-H7

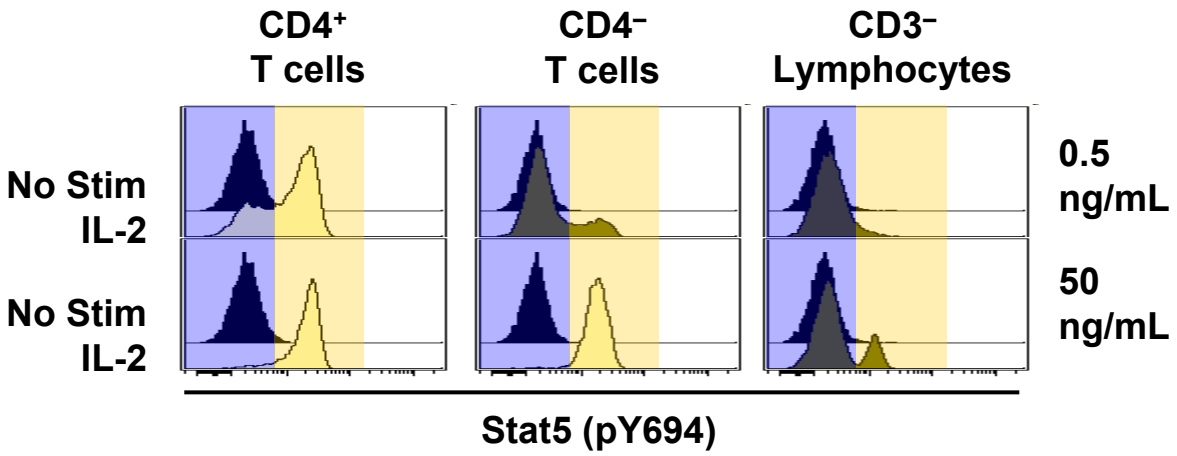
Perm Buffers	Fixation Buffers	Protocols
BD™ Phosflow Perm/Wash Buffer I (557885)		Detergent Method
BD™ Phosflow Perm Buffer II (558052)		Mild Alcohol Method
BD™ Phosflow Perm Buffer III (558050)		Harsh Alcohol Method
BD™ Phosflow Perm Buffer IV (560746)		Harsh Detergent Method

Specificity								BD Horizon™ V450	Pacific Blue™	AmCyan	BD Horizon™ V500	Alexa Fluor® 488	FITC	PE	PE-Texas Red®	PE-Cy™5	PerCP	PerCP-Cy™5.5	PE-Cy™7	PE	PE-Cy™5	PE-Cy™7	APC	Alexa Fluor® 647	Alexa Fluor® 700	APC-Cy™7	APC-H7	
Target	Clone △	Human	Mouse	Surface	Intracellular	Source	Protocol																						
CD3e	145-2C11		♦	♦		BALB/c SP	Std					♦	♦	♦		◊	♦	♦	♦	◊	◊	◊	♦			♦		View
CD3	17A2		♦	♦		BALB/c SP	Std	♦					♦	♦		◊		♦	♦	◊	◊	◊		♦	◊	♦		View
p38 MAPK (pT180/pY182)	36/p38 (pT180/pY182)	♦			♦	Whole Blood	Std		♦			♦		♦				♦	♦	◊		◊		♦				View
p38 MAPK (pT180/pY182)	36/p38 (pT180/pY182)	♦			♦	PBMC	Std		♦			♦		♦				♦	♦	◊		◊		♦				View
p38 MAPK (pT180/pY182)	36/p38 (pT180/pY182)		♦		♦	BALB/c SP	Std		♦			♦		♦				♦	♦	◊		◊		♦				View
Stat4 (pY693)	38/p-Stat4	♦			♦	PBMC	Std					♦		♦				♦		◊				♦				View
Stat4 (pY693)	38/p-Stat4	♦			♦	Whole Blood	Std					♦		♦				♦		◊				♦				View

BD

Designing a Multicolor Phosflow Staining Panel

Heterogeneous Threshold for IL-2 Responsiveness within Lymphocyte Subpopulations in Human Whole Blood

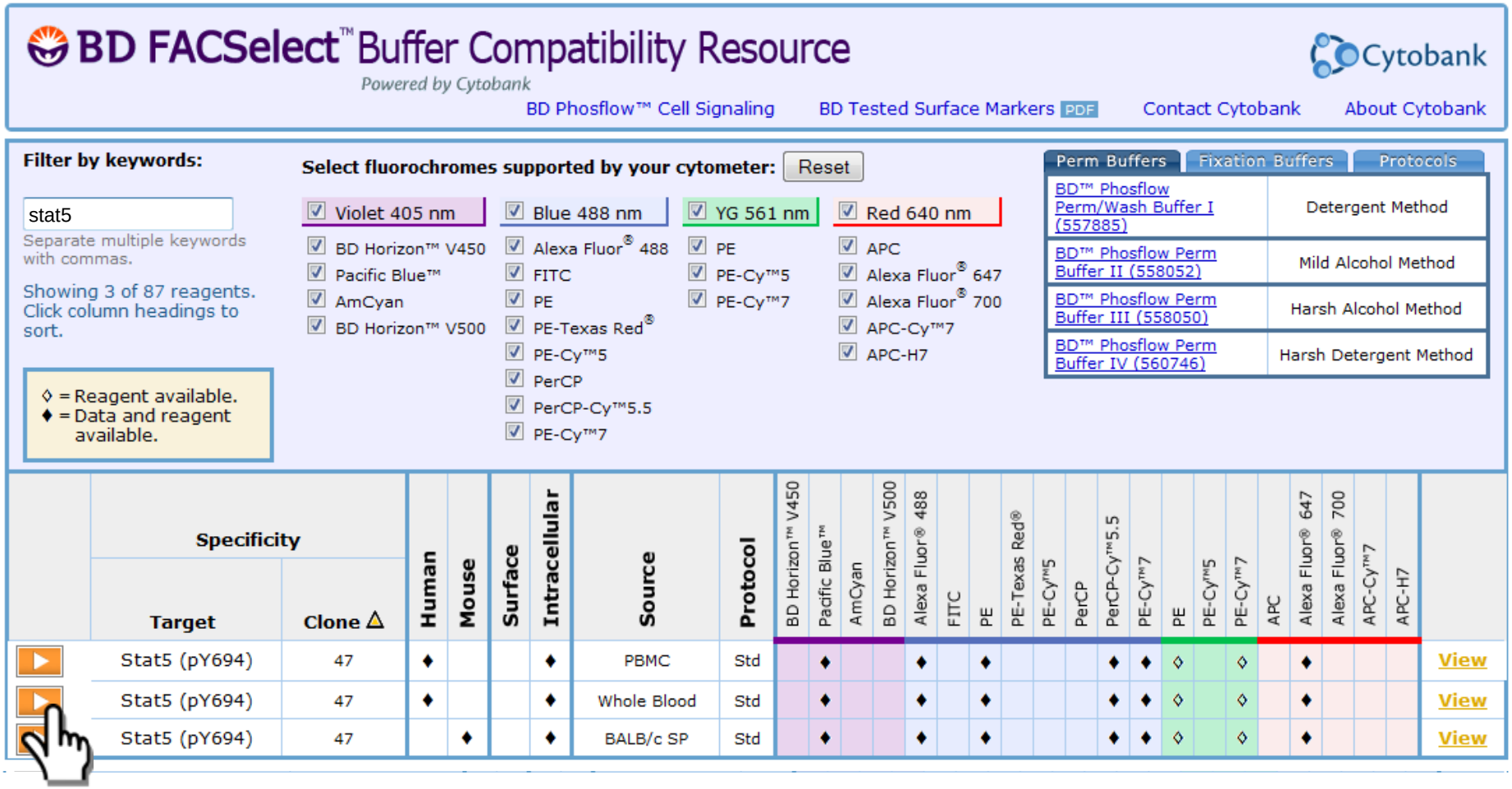


Does IL-2 responsiveness differ between naïve, effector, and memory T cells?

Need to simultaneously stain CD3, CD4, CD45RA, CD45RO, T-bet, and Stat5 (pY694) in IL-2–stimulated human whole blood cells

BD

Step 1: Check Buffer Compatibility for Intracellular Antibodies



	Specificity																												
	Target	Clone △	Human	Mouse	Surface	Intracellular	Source	Protocol	BD Horizon™ V450	Pacific Blue™	AmCyan	BD Horizon™ V500	Alexa Fluor® 488	FITC	PE	PE-Texas Red®	PE-Cy™5	PerCP	PerCP-Cy™5.5	PE-Cy™7	PE	PE-Cy™5	PE-Cy™7	APC	Alexa Fluor® 647	Alexa Fluor® 700	APC-Cy™7	APC-H7	
	Stat5 (pY694)	47	♦			♦	PBMC	Std		♦			♦		♦				♦	♦	◊		◊		♦				View
	Stat5 (pY694)	47	♦			♦	Whole Blood	Std		♦			♦		♦				♦	♦	◊		◊		♦				View
	Stat5 (pY694)	47		♦		♦	BALB/c SP	Std		♦			♦		♦				♦	♦	◊		◊		♦				View

BD

Stat5 (pY694) Antibody Works Well with Perm III or Perm IV (0.5x or 1x)

Stat5 (pY694) (47)

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Back to FACSelect

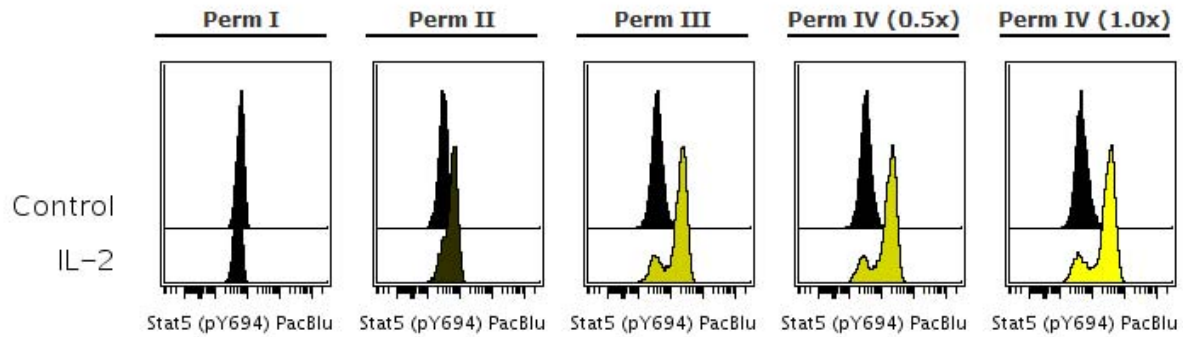
About Stat5 (pY694) (47)

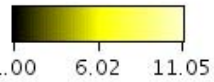
Protein Name: Stat5 (pY694)
Clone: 47
Isotype: IgG1
Reactive species: Human, Mouse
Host species: Mouse
Protocol: details
Experiment Cell Source: Human Whole Blood
Cytometer used: FACSCantoII

Conjugates Shown

Pacific Blue™ [BD]
Alexa Fluor® 488 [BD]
PE [BD]
PerCP-Cy5.5 [BD]
PE-Cy™7 [BD]
Alexa Fluor® 647 [BD]

Stat5 (pY694) PacBlue





Calculated Fold of Medians by First Row using X-Axis channel(s): Use Panel/Channel Values

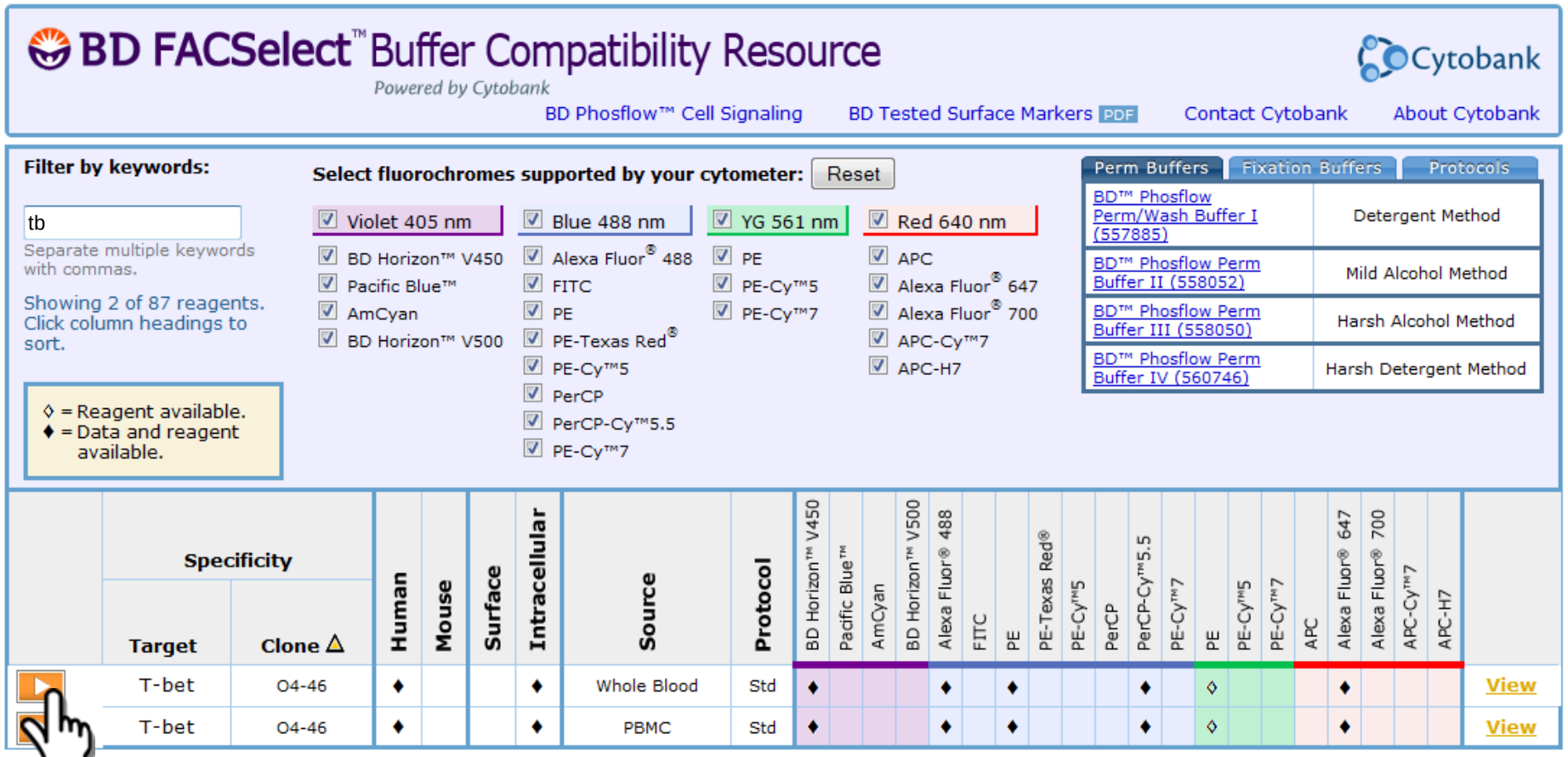
	Perm I	Perm II	Perm III	Perm IV (0.5x)	Perm IV (1.0x)
Control	1.0	1.0	1.0	1.0	1.0
IL-2	0.84	1.93	5.06	5.21	6.15

View in Cytobank Jump to Gating Hierarchy Back to Top

~~Perm I~~
~~Perm II~~
Perm III
Perm IV 0.5x
Perm IV 1.0x

BD

Step 1: Check Buffer Compatibility for Intracellular Antibodies



T-bet Antibody Also Works with Perm III or Perm IV (0.5x or 1x)

T-bet (O4-46)

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Back to FACSelect

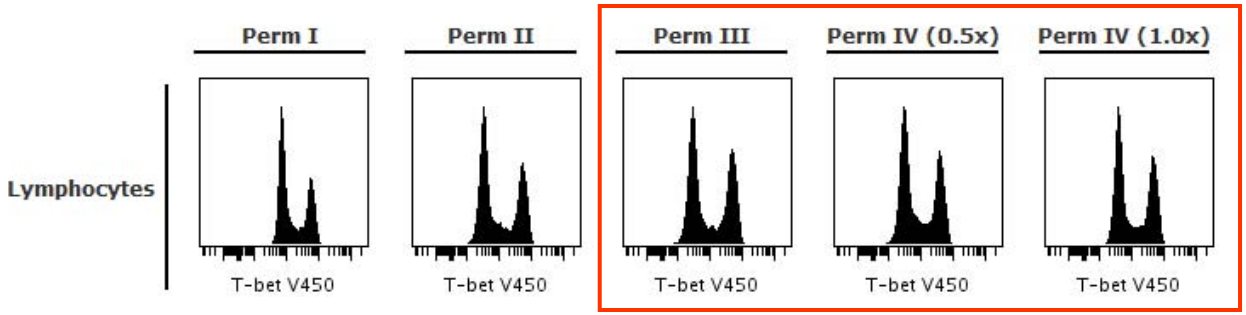
About T-bet (O4-46)

Protein Name: T-bet
Clone: O4-46
Isotype: IgG1, k
Reactive species: Human, Mouse
Host species: Mouse
Protocol: details
Experiment Cell Source: Human Whole Blood
Cytometer used: FACSCantoII

Conjugates Shown

V450 [BD]
Alexa Fluor® 488 [BD]
PE [BD]
PerCP-Cy5.5 [BD]
Alexa Fluor® 647 [BD]

T-bet V450



Calculated Raw values of statistic using X-Axis channel(s): Use Panel/Channel Values

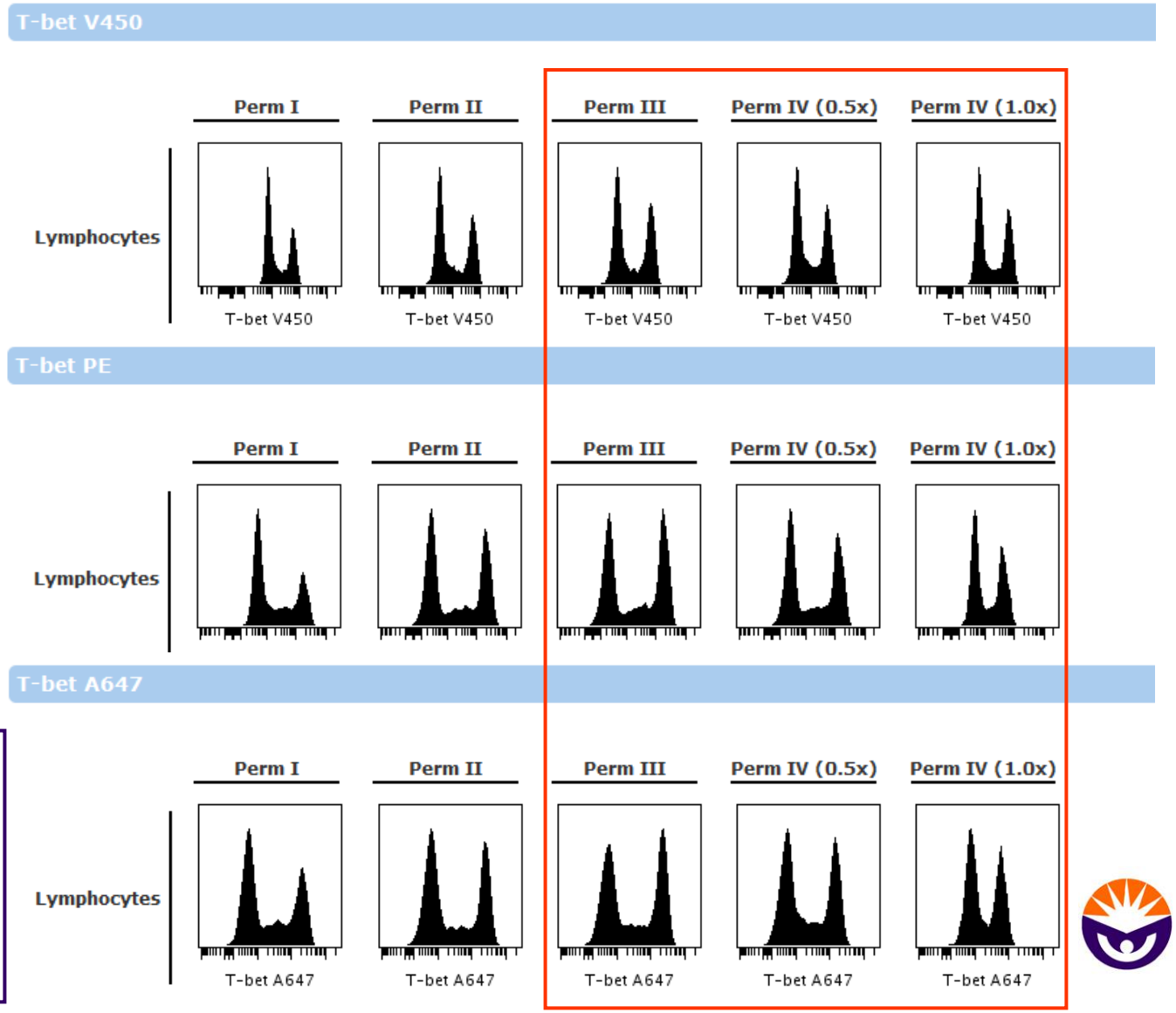
	Perm I	Perm II	Perm III	Perm IV (0.5x)	Perm IV (1.0x)
Lymphocytes	2.6	3.48	3.57	3.28	3.16

View in Cytobank Jump to Gating Hierarchy Back to Top

~~Perm I~~
~~Perm II~~
Perm III
Perm IV 0.5x
Perm IV 1.0x

BD

Best T-bet Resolution with Brightest Fluorophores and Perm III or IV (0.5x)



T-bet on PE or Ax647

~~Perm I~~

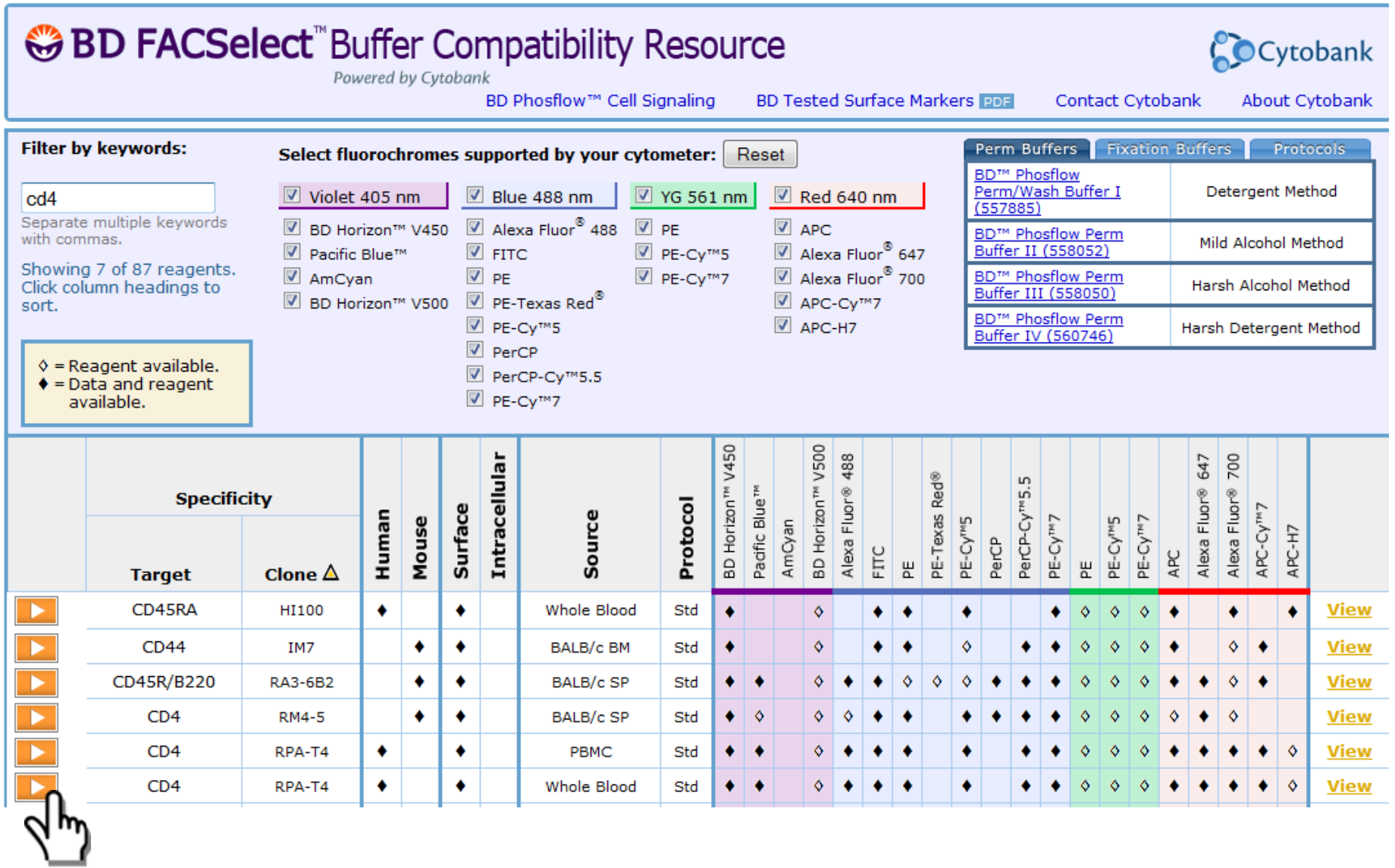
~~Perm II~~

Perm III

Perm IV 0.5x

Perm IV 1.0x?

Step 2: Check Buffer Compatibility for Surface Marker Antibodies



	Specificity		Human	Mouse	Surface	Intracellular	Source	Protocol	BD Horizon™ V450	Pacific Blue™	AmCyan	BD Horizon™ V500	Alexa Fluor® 488	FITC	PE	PE-Texas Red®	PE-Cy™5	PerCP	PerCP-Cy™5.5	PE-Cy™7	PE	PE-Cy™5	PE-Cy™7	APC	Alexa Fluor® 647	Alexa Fluor® 700	APC-Cy™7	APC-H7	
	Target	Clone Δ																											
▶	CD45RA	HI100	♦		♦		Whole Blood	Std	♦			◊		♦	♦		♦			♦	◊	◊	◊	♦		♦		♦	View
▶	CD44	IM7		♦	♦		BALB/c BM	Std	♦			◊		♦	♦		◊		♦	♦	◊	◊	◊	♦		◊	♦		View
▶	CD45R/B220	RA3-6B2		♦	♦		BALB/c SP	Std	♦	♦		◊	♦	♦	◊	◊	◊	♦	♦	♦	◊	◊	◊	♦	♦	◊	♦		View
▶	CD4	RM4-5		♦	♦		BALB/c SP	Std	♦	◊		◊	◊	♦	♦		♦	♦	♦	♦	◊	◊	◊	◊	♦	◊			View
▶	CD4	RPA-T4	♦		♦		PBMC	Std	♦	♦		◊	♦	♦	♦		♦		♦	♦	◊	◊	◊	♦	♦	♦	♦	◊	View
▶	CD4	RPA-T4	♦		♦		Whole Blood	Std	♦	♦		◊	♦	♦	♦		♦		♦	♦	◊	◊	◊	♦	♦	♦	♦	◊	View

CD4 Clone RPA-T4 is Not Compatible with Perm Buffer IV

CD4 (RPA-T4)

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Back to FACSelect

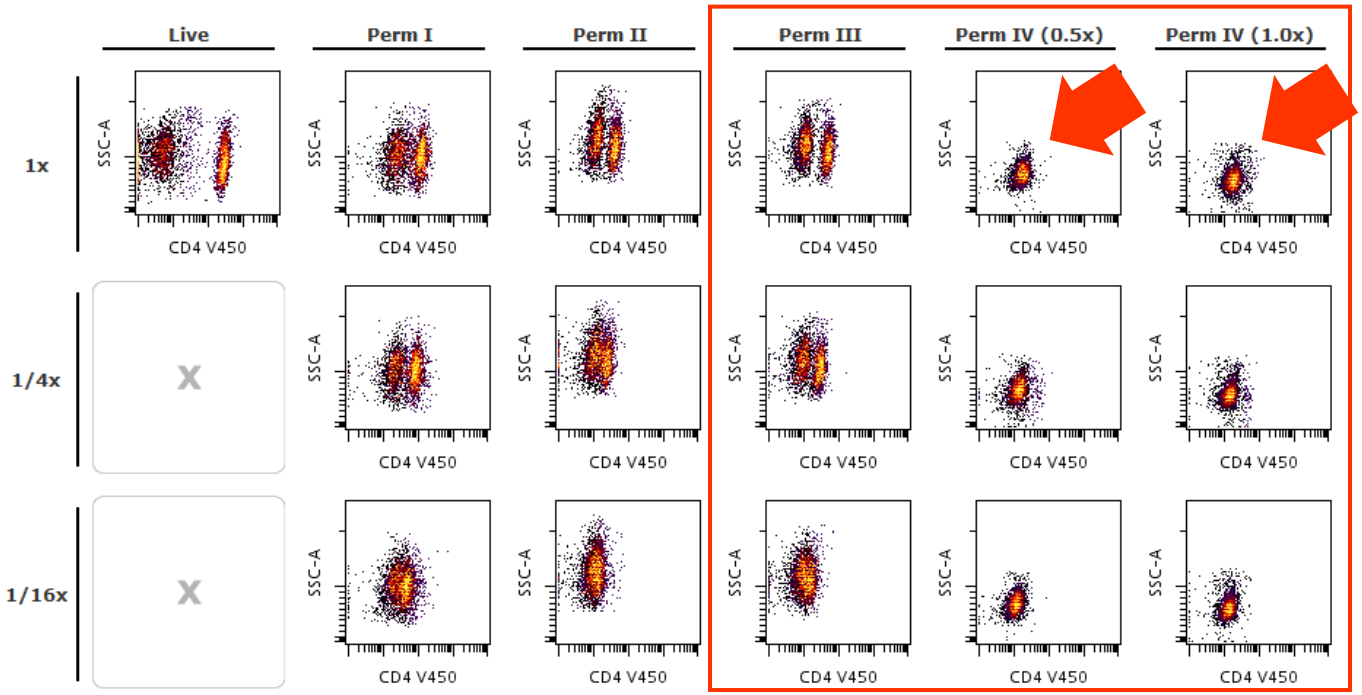
About CD4 (RPA-T4)

Protein Name: CD4
Clone: RPA-T4
Isotype: IgG1, k
Reactive species: Human
Host species: Mouse
Protocol: details
Experiment Cell Source: Human Whole Blood
Cytometer used: LSRII

Conjugates Shown

V450 [BD]
Pacific Blue™ [BD]
Alexa Fluor® 488 [BD]
FITC [BD]
PE [BD]
PE-Cy™5 [BD]
PerCP-Cy5.5 [BD]
PE-Cy™7 [BD]
APC [BD]
Alexa Fluor® 647 [BD]
Alexa Fluor® 700 [BD]
APC-Cy™7 [BD]

CD4 V450

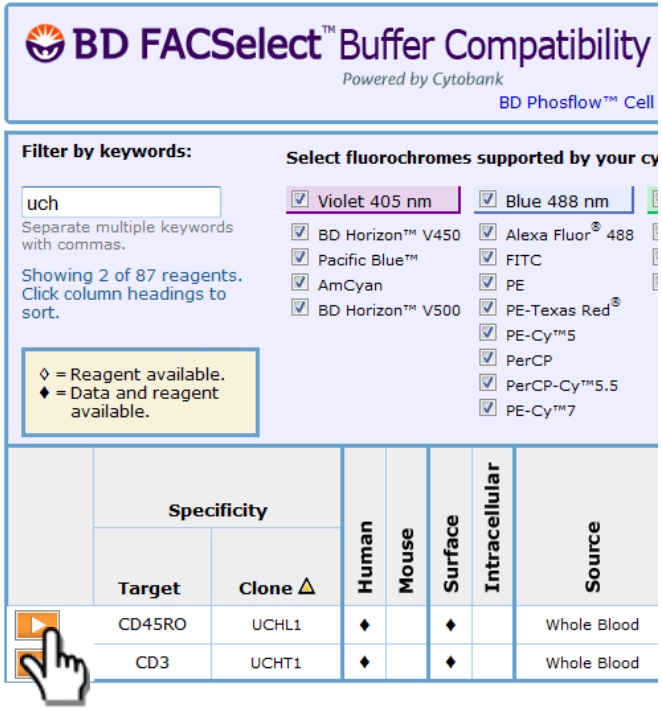


T-bet on PE or Ax647

~~Perm I~~
~~Perm II~~
Perm III
~~Perm IV 0.5x~~
~~Perm IV 1.0x~~ ?

Step 3: Confirm Compatibility of All Antibodies with Selected Buffer

Specificity	Clone	Compatible with Perm Buffer III?
Stat5 (pY694)	47	✓
T-bet	O4-46	✓
CD3	UCHT1	✓
CD4	RPA-T4	✓
CD45RA	HI100	✓
CD45RO	UCHL1	✓



T-bet on PE or Ax647

~~Perm I~~

~~Perm II~~

Perm III

~~Perm IV 0.5x~~

~~Perm IV 1.0x~~

Note: If some surface markers are incompatible with the chosen buffer system, alternative staining protocols may be useful. Be aware of fluorophore choice considerations.

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Step 4: Select an Appropriate Conjugate for Each Antibody

Specificity	Clone	Compatible with Perm Buffer III?	Fluorophore	Optimal Concentration
Stat5 (pY694)	47	✓	Ax647	
T-bet	O4-46	✓	PE	
CD3	UCHT1	✓	Ax488	
CD4	RPA-T4	✓	PE-Cy7	
CD45RA	HI100	✓	V450	
CD45RO	UCHL1	✓	PerCP-Cy5.5	

T-bet on PE or Ax647

~~Perm I~~

~~Perm II~~

Perm III

~~Perm IV 0.5x~~

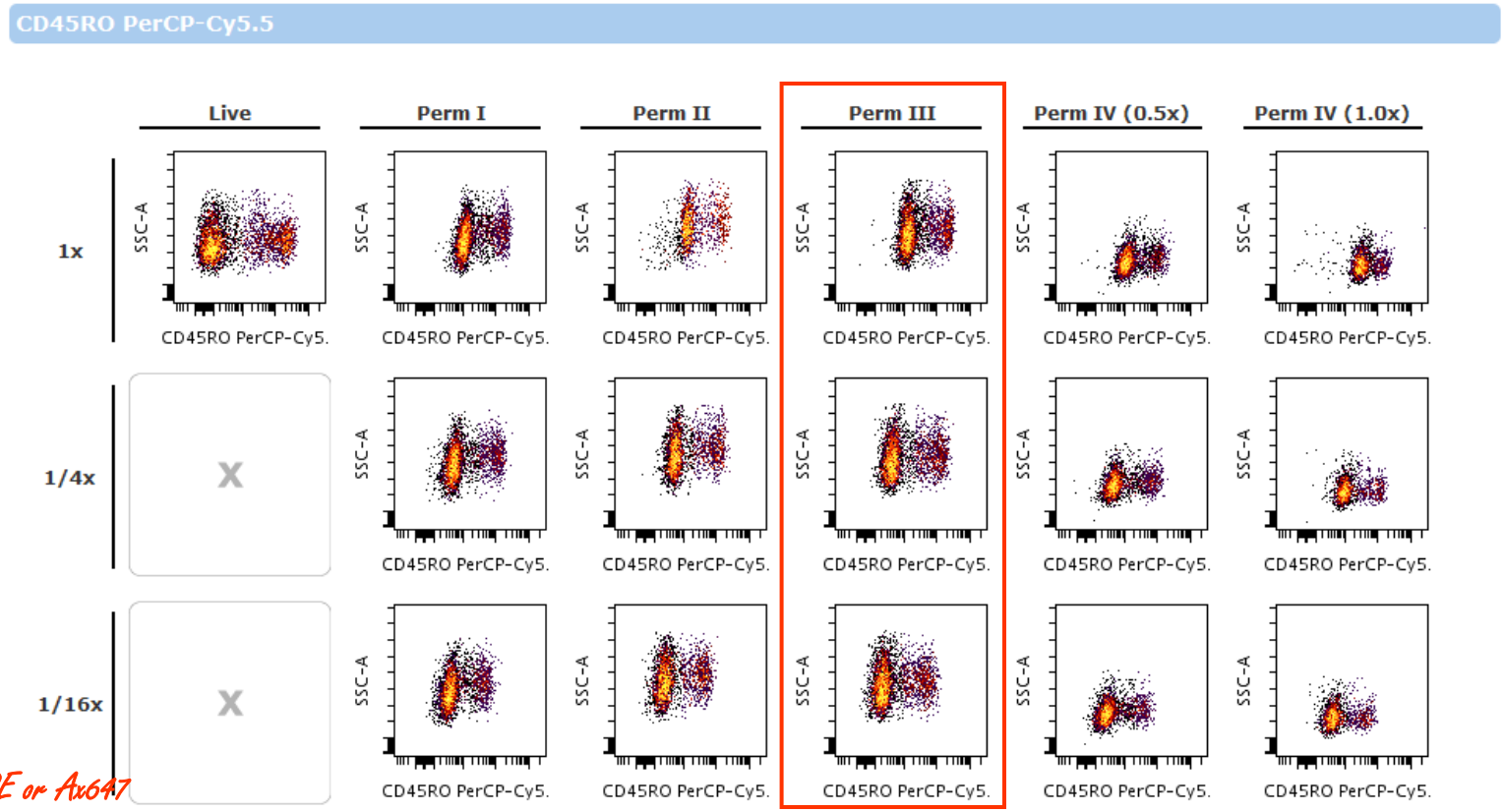
~~Perm IV 1.0x~~

General Principles for Panel Design:

- Brightest fluorophores should be used for phospho-specific antibodies and other dim or important stains
- Try to avoid spectral overlap in channels used for phosphoprotein detection

BD

Step 5: Identify the Optimal Concentration for Each Antibody



T-bet on PE or Ax647

~~Perm I~~

~~Perm II~~ Use CD45RO

Perm III PerCP-Cy5.5

~~Perm IV 0.5x~~ @ ¼ x

~~Perm IV 1.0x~~

Calculated Raw values of statistic using X-Axis channel(s): Use Panel/Channel Values

	Live	Perm I	Perm II	Perm III	Perm IV (0.5x)	Perm IV (1.0x)
1x	5.93	3.28	3.71	3.32	2.88	2.2
1/4x	X	3.7	3.65	4.01	3.45	2.96
1/16x	X	3.15	3.54	3.84	3.28	3.04

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Step 5: Identify the Optimal Concentration for Each Antibody

Specificity	Clone	Compatible with Perm Buffer III?	Fluorophore	Optimal Concentration
Stat5 (pY694)	47	✓	Ax647	Test Size
T-bet	O4-46	✓	PE	Test Size
CD3	UCHT1	✓	Ax488	1x
CD4	RPA-T4	✓	PE-Cy7	1x
CD45RA	HI100	✓	V450	1x
CD45RO	UCHL1	✓	PerCP-Cy5.5	¼ x

T-bet on PE or Ax647

~~Perm I~~

~~Perm II~~

Perm III

~~Perm IV 0.5x~~

~~Perm IV 1.0x~~

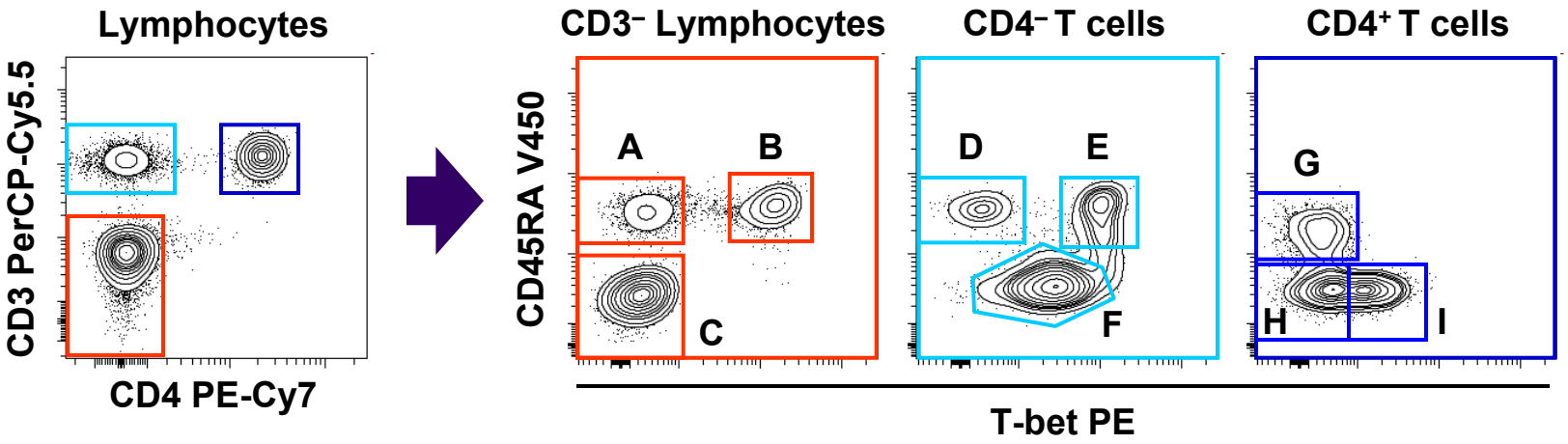
Use CD45RO PerCP-Cy5.5 @ ¼ x

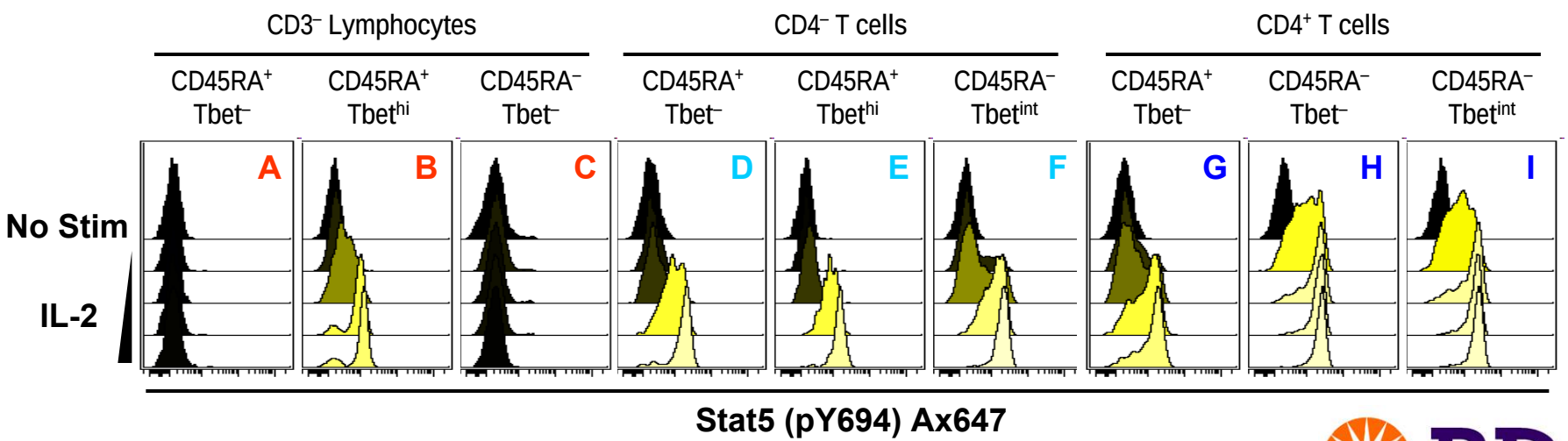
General Principles for Panel Design:

- Brightest fluorophores should be used for phospho-specific antibodies and other dim or important stains
- Try to avoid spectral overlap in channels used for phosphoprotein detection

BD

Step 6: Stimulate, Fix, Perm, Stain, and Get Great Data





BD

Helpful Resources

BD FACSelect™ Buffer Compatibility Resource

cytobank.org/facselect

BD Phosflow™ Website

bdbiosciences.com/phosflow

BD FACSelect™ Multicolor Panel Designer

bdbiosciences.com/paneldesigner

Manage, analyze, and share flow cytometry data over the web with Cytobank

cytobank.org

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23-13813-00

Thank You!

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