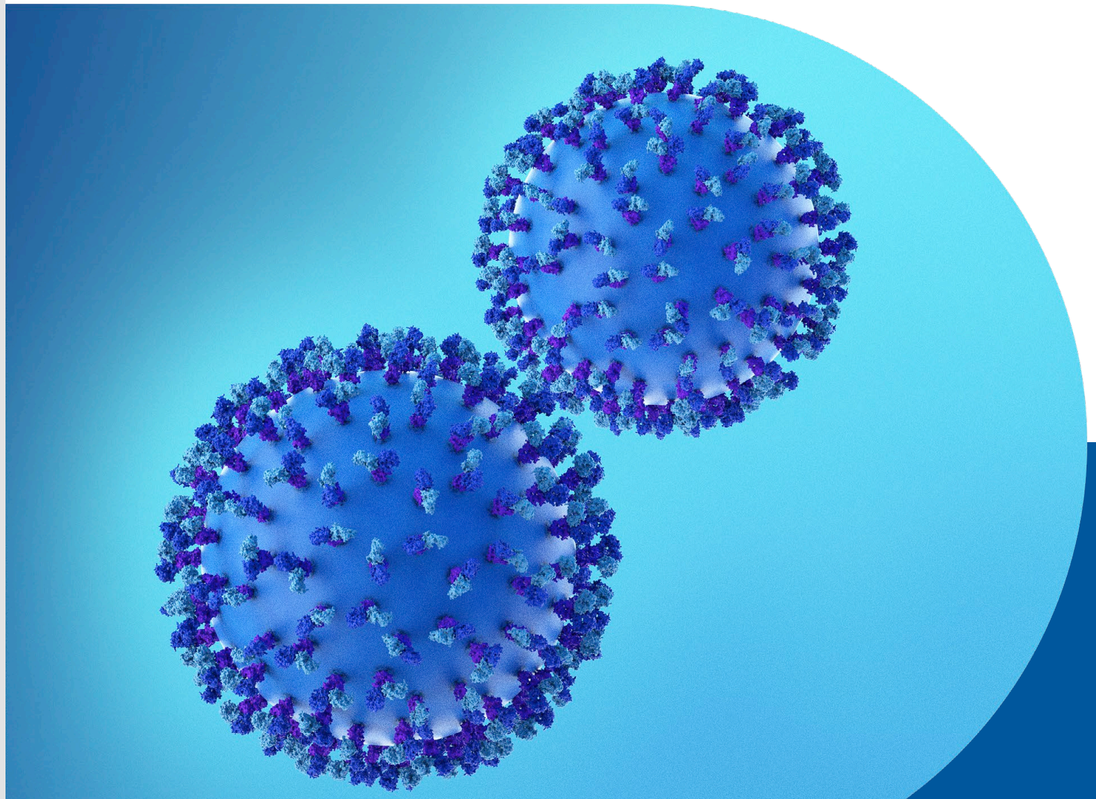
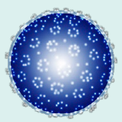


BD Horizon™ Human T Cell Backbone Panel

Expand your T cell research with confidence

BD



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The development of larger multicolor panels has become critical to ensure deeper T cell characterization. However, expansion of existing flow cytometry panels is more challenging than simply adding new fluorochromes and markers. The introduction of spillover spread may compromise panel resolution, ultimately requiring a complete redesign of a new panel. This affects assay cost and development time but most importantly limits your ability to define cell biology more in depth.

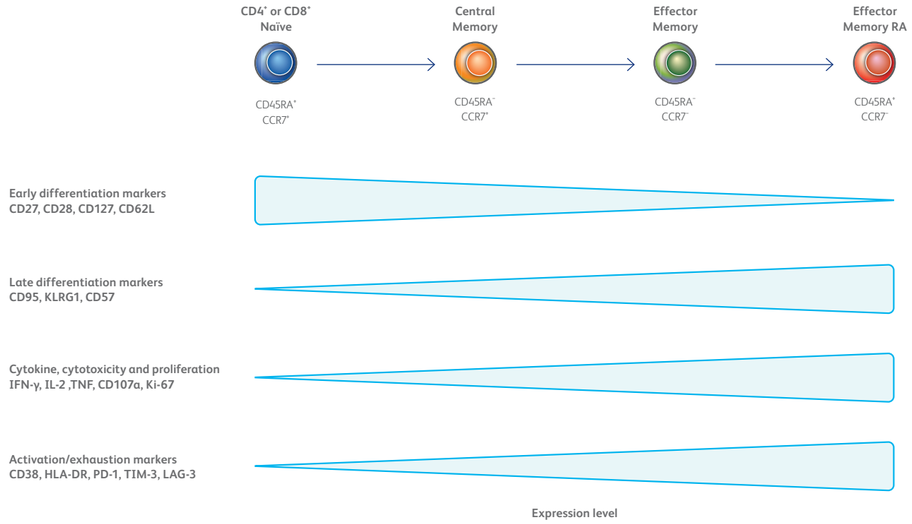
What if you could focus more on expanding your T cell research and worry less about panel design and resolution loss? Now you can with the BD Horizon™ Human T Cell Backbone Panel. This pre-optimized panel is strategically designed to enable addition of up to five markers using defined fluorochromes with minimal panel design effort, while maintaining optimal resolution.

The BD Horizon™ Human T Cell Backbone Panel contains five individual vials of fluorochrome conjugated antibodies against T cell core markers, CD3, CD4, CD8, CD45RA, CD197 (CCR7), conventionally used to assess T cell maturation and identify naïve, central memory (CM), effector memory (EM) and effector memory RA (EMRA) subsets.[1,2] The kit also contains the BD Horizon™ Brilliant Stain Buffer for optimal performance.

Panel features

- Clear resolution of five core markers defining major naïve and memory subsets of human $CD4^+$ and $CD8^+$ T cells
- Strategically designed to be complemented with defined fluorochromes with no resolution impact into both the backbone and drop-ins
- Verified protocols to ensure optimal performance
- Consistent performance across instruments with different optical configurations
- Compatible with buffers and protocols used for intracellular detection of cytokines, transcription factors or phosphorylated proteins
- Verified flexibility enabling deeper investigation of different facets of T cell biology (activation, differentiation, polarization, Treg immunophenotype)

T cell maturation



While this minimal combination of surface markers may be sufficient for high-level characterization of T cell subsets, additional markers are required for a deeper immunophenotypic and functional resolution of these highly heterogeneous cells.[3,4]

Furthermore, the BD Horizon™ Human T Cell Backbone Panel could be used as a starting point for a deep dive into, for example, $CD4^+$ T helper cells and further identification of regulatory T cells through the addition of specific drop-ins.

References

1 Hamann D, Baars PA, Rep MH, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. *J Exp Med.* 1997;186(9):1407-1418. doi: 10.1084/*J Exp Med.*11-1997

2 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. et al. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature.* 1999;401:708–712. doi:10.1038/*Nature.*10-1999

3 Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: human memory T-cell subsets. *Eur J Immunol.* 2013;43(11):2979-2809. doi: 10.1002/eji. 10-2013

4 Larbi A, Fulop T. From "truly naïve" to "exhausted senescent" T cells: when markers predict functionality. *Cytometry A.* 2014;85(1):25-35. doi: 10.1002/Cyto.a.22351.

Learn more about the BD Horizon™ Human T Cell Backbone Panel

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The BD Horizon™ Human T Cell Backbone Panel is strategically designed to be complemented with up to five recommended drop-in fluorochromes of choice, depending on instrument configuration, with minimal panel design effort and resolution loss.

Backbone panel markers (Cat. No. 568263)

Marker	Clone	Fluorochrome
CD3	UCHT1	BV510
CD4	SK3	BV786
CD8	RPA-T8	R718
CD45RA	HI100	PE-Cy7
CD197 (CCR7)	2-L1-A	BV711

Example of tested and recommended drop-in fluorochromes

Laser	Drop-in fluorochromes	Relative brightness ranking*
Violet 405 nm	BV421 V450	Very Bright Dim
Blue 488 nm	FITC BB515 AF488	Moderate Very Bright Moderate
Blue 488 nm	PE	Bright
Yellow-green 561 nm	PE RY586	Very Bright Very Bright
Red 640 nm	APC AF647	Bright Bright
UV 355 nm	BUV395	Moderate

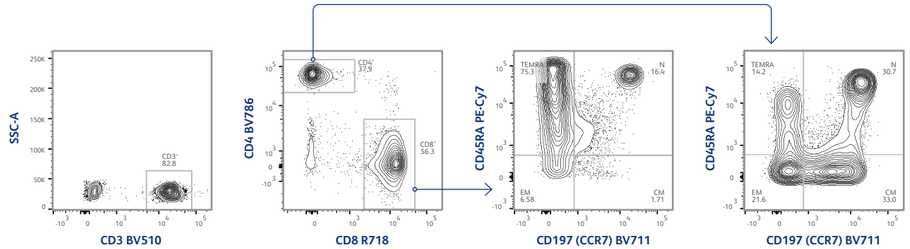
* Relative Brightness Ranking provides general guidance with respect to the relative capability of different fluorochromes to resolve dimly stained populations; it is not a representation of absolute fluorescence. Rankings were determined by comparing the stain index (resolution) of cells stained with multiple formats on several clones run on a variety of flow cytometers. Many factors can influence the relative fluorochrome/reagent performance on a given instrument, including laser power, PMT voltage, optical filters, antibody clone and biological sample.

Disclaimers

1 Additional fluorochromes with highly similar spectral profiles to the one tested here (e.g., V450 and Pacific Blue™) may be used as recommended drop-ins.
2 Fluorescent proteins or dyes (e.g., GFP, DAPI, CFSE) detected in the same channels, but with spectral profiles different from the recommended drop-in fluorochromes, should be further tested for their resolution impact.
3 The backbone panel can be complemented with more than the recommended fluorochrome drop-ins, although resolution maintenance cannot be guaranteed.
4 The BD Horizon RealYellow™ (RY) 586 Fluorochrome can be used as an alternative to PE when excited off the yellow-green laser. Simultaneous use of PE off the blue laser and RY586 off the yellow-green laser is not recommended.

Four fundamental requirements were tested to verify the performance of the backbone panel and the ability to easily add recommended fluorochromes without introduction of meaningful spillover spread.

1. The backbone panel clearly resolves major T cell subsets



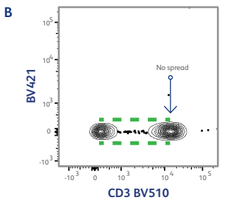
Human peripheral blood mononuclear cells (PBMCs) were stained with the BD Horizon™ Human T Cell Backbone Panel and acquired on a 3-laser, 12-color BD FACSLyric™ Flow Cytometer. Performance was also tested on the 4-laser BD FACSymphony™ A1, 5-laser BD LSRFortessa™ X-20, BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of T cell subsets was observed irrespective of the instrument utilized (not shown).

2. The backbone panel does not impact resolution of the recommended drop-in fluorochromes

A

Total spread matrix		Into drop-ins				
		BV421	FITC	PE	APC	BV605
From backbone fluorohromes	BV510	13	8	0	0	290
	BV786	12	0	0	33	15
	R718	32	0	7	14	0
	PE-Cy7	18	8	25	18	10
	BV711	0	0	0	69	14

B



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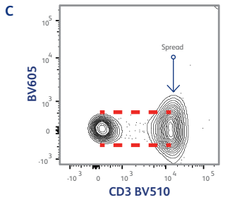
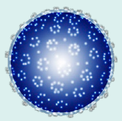
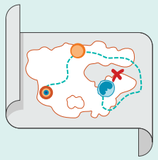


A) Representative total spread matrix calculated on FlowJo™ v10.8.1 Software using the BD Horizon™ Human T Cell Backbone Panel reagents. Negligible spillover spread is introduced into the channels used for conventional detection of the recommended fluorochrome drop-in BV421, FITC, PE and APC as compared to BV605, which is not recommended as a drop-in. **B)** Representative visualization of the lack of spillover spread from CD3 BV510 into BV421 channel. **C)** BV605 is shown here as a drop-in that would be impacted by CD3 BV510.



Learn more about the BD Horizon™ Human T Cell Backbone Panel



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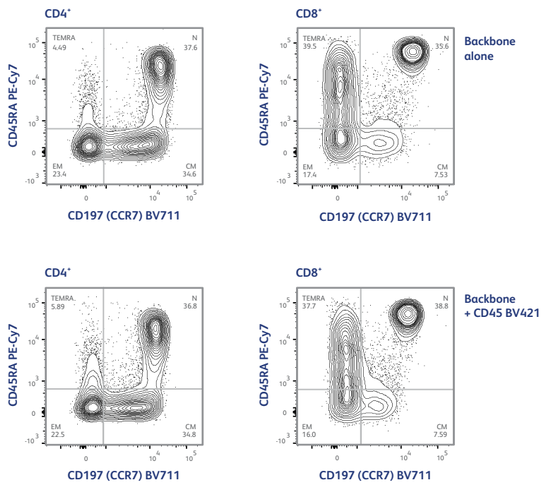
3. The drop-in fluorochromes do not impact resolution of the backbone panel

A



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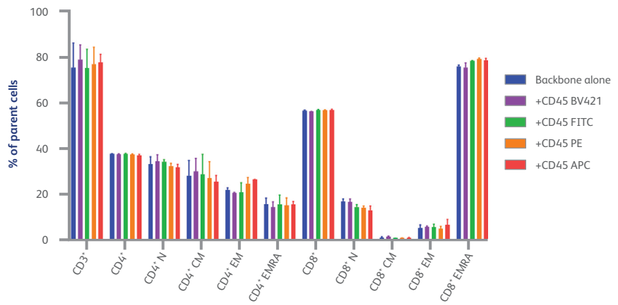


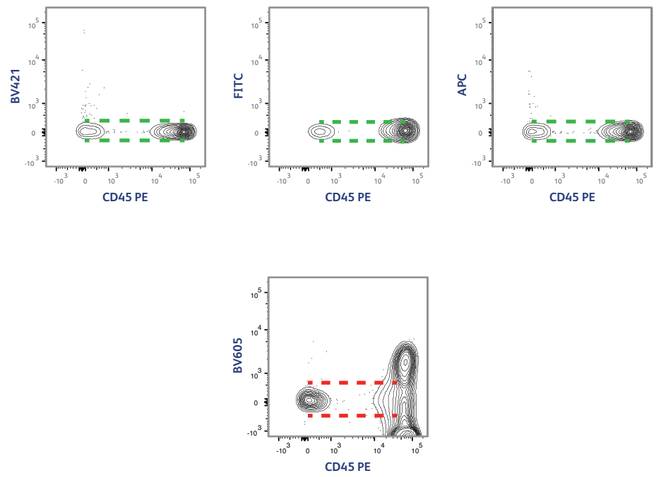
A) Resolution of representative T cell populations before and after addition of BV421 mouse anti-human CD45 antibody.
B) Quantification of T cell subsets before and after addition of individual examples of mouse anti-human conjugated antibodies. No impact to resolution or quantitation was observed upon addition of the recommended drop-ins, despite CD45 being highly expressed and co-expressed by all T cells. Similar results were obtained when cells were individually stained with CD45 conjugated to V450, BB515, AF488, RY586 or AF647 (not shown).

4. The drop-in fluorochromes do not impact each other

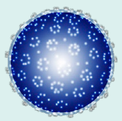
A

Total spread matrix		Into drop-in channels				
		BV421	FITC	PE	APC	BV605
From drop-in fluorohromes	BV421	0	0	0	0	52
	FITC	0	0	0	0	54
	PE	0	0	0	0	1405
	APC	0	0	0	0	15

B



A) Representative total spread matrix. The recommended drop-ins BV421, FITC, PE and APC are spatially separated and do not introduce spillover spread into each other's channels. **B)** Representative visualization of the lack of spillover spread from PE into the other recommended fluorochromes. **C)** BV605 is shown here as a drop-in fluorochrome that may be impacted by the recommended drop-in PE.



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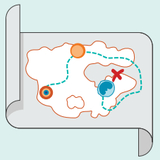

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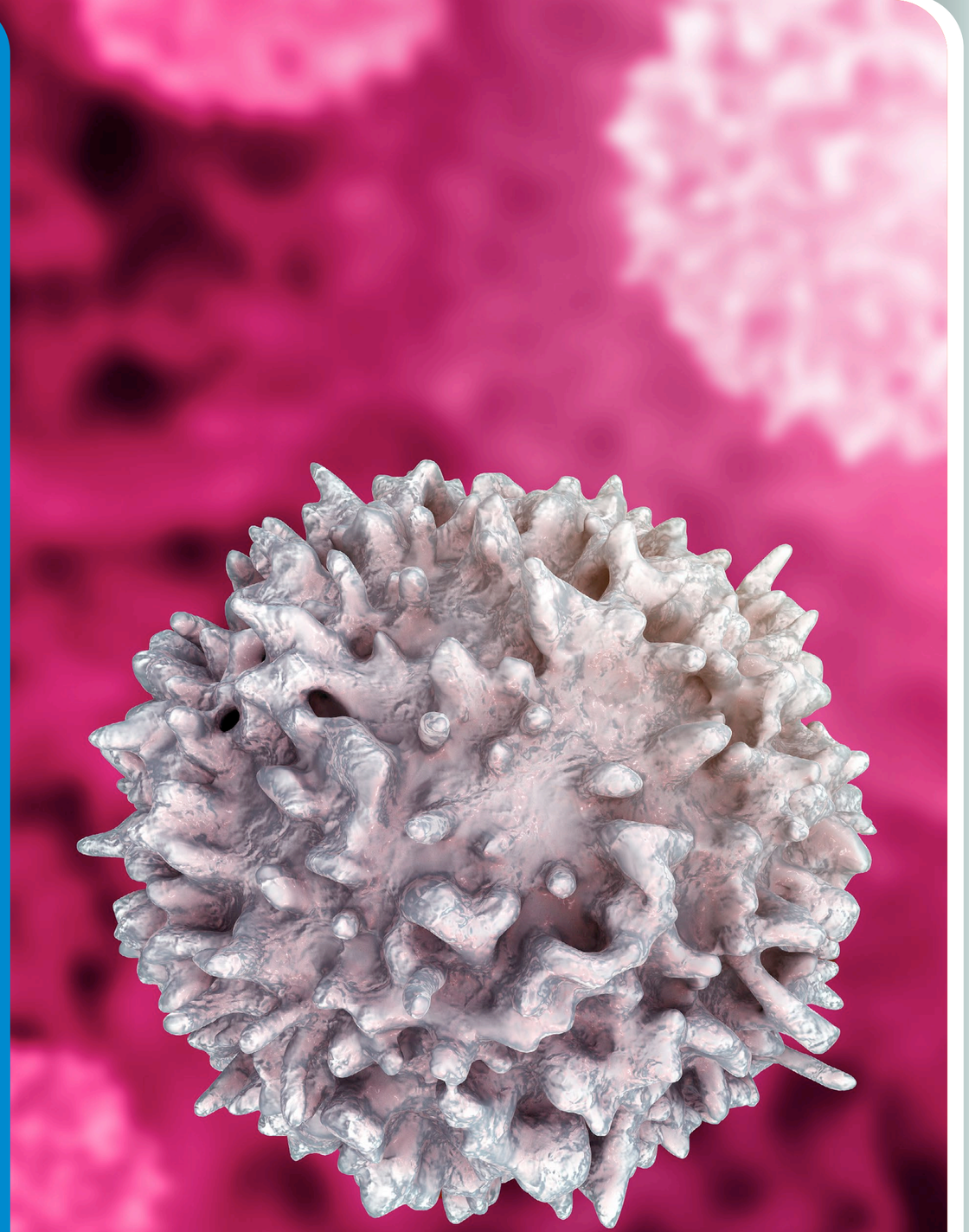


tips&tricks

Perform RBC lysis post antibody staining to avoid potential impact on antigen integrity and antibody binding.

Perform enrichment via negative selection (magnetic depletion of unwanted cells) to minimize perturbation of cell populations of interest.

Some buffers for RBC lysis (BD FACS™ Lysing Solution) contain a fixative buffer, whereas others (BD Pharm Lyse™ Lysing Buffer) do not. The potential impact on antigen integrity and the compatibility with viability stains need to be taken into consideration when using RBC lysis solutions containing a fixative buffer.

Optional Protocols

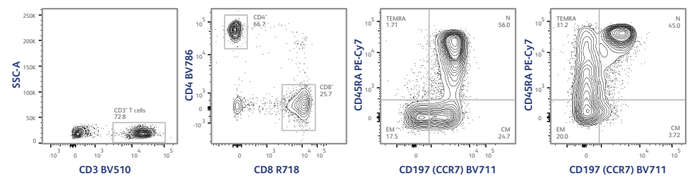
Sample type

The performance of the BD Horizon™ Human T Cell Backbone Panel was verified on human lysed whole blood, fresh and frozen peripheral mononuclear PBMCs, enriched, cultured and activated T cells isolated from healthy donors.

Sample type	Target Populations	Method	Application Examples
Lysed whole blood (LWB)	Mononucleated and polymorphonucleated cells	Red blood cell (RBC) lysis	Broad and/or high-level immunophenotyping of major immune cells abundantly expressed
Peripheral blood mononuclear cells (PBMCs)	Mononucleated cells	Ficoll gradient separation	Deeper immunophenotyping of major immune cells and rare subsets thereof
Enriched T cells	T cell subsets	Magnetic isolation	Immunophenotyping of T cells after ex vivo culture and activation

Performance of BD Horizon™ HumanT Cell Backbone Panel on two different sample types.

A



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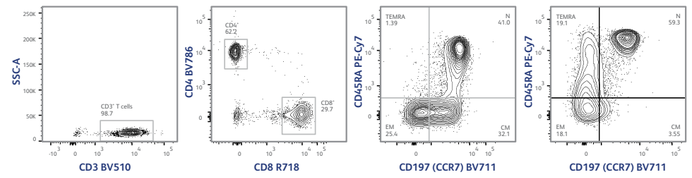
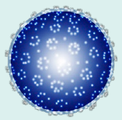


A) Human PBMCs. **B)** Magnetically enriched T cells cultured for 2 days in complete medium at 37 °C.



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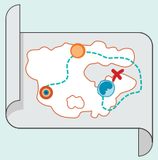

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tips&tricks

Use the provided BD Horizon™ Brilliant Stain Buffer to ensure optimal performance.

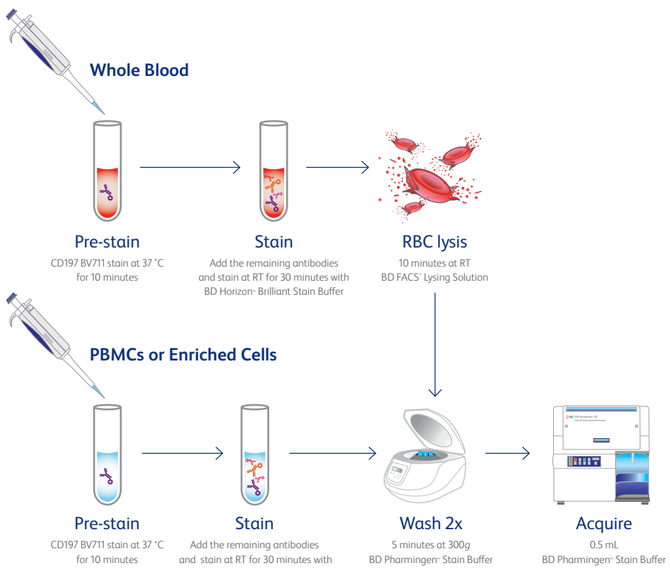
The pre-stain protocol for optimal performance of the CD197 BV711 antibody can be used to improve resolution of any other chemokine receptor.

Resting of thawed PBMCs in complete medium may be required to improve CD197 resolution.

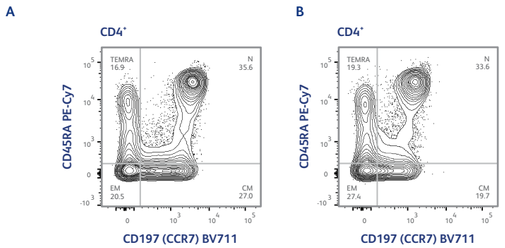
BD Human Fc Block™ Reagent should be added when nonspecific Fc binding is of concern, such as when using antibodies with IgG2a isotype.

Antibody staining

Different workflows can be used to stain cells with the BD Horizon™ Human T Cell Backbone Panel, depending on the type of sample used.



For optimal resolution of the chemokine receptor CD197 (CCR7), it is recommended to pre-stain with the anti-human CD197 BV711 antibody at 37 °C for 10 minutes before addition of the remaining antibodies and incubation at RT for 30 minutes.



A) Human PBMCs were pre-stained with CD197 BV711 antibody at 37 °C for 10 minutes. The remaining antibodies were then added and incubation continued at RT for an additional 30 minutes. **B)** The same sample was stained with all the antibodies at RT for 30 minutes. Pre-incubation of anti-CD197 antibody at 37 °C for 10 minutes improves resolution. Prolonged incubation of all antibodies for 40 minutes at 37 °C can slightly improve resolution (not shown).

tips&tricks

Make sure the signal of the compensation control is as bright or brighter than the experimental sample.

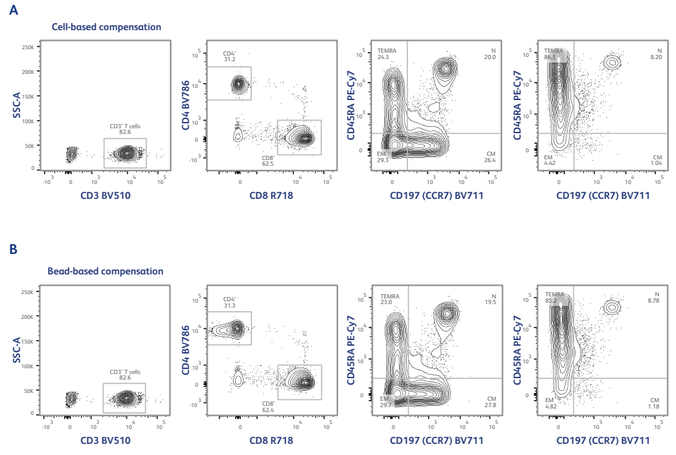
Treat the compensation controls exactly as the experimental sample, e.g., add BD Horizon™ Brilliant Stain Buffer, fixation and permeabilization buffer to control samples.

Do not change fluorescence detector voltage or gain after acquisition of the compensation controls.

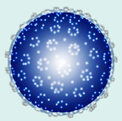
Always test the accuracy of compensation when using cells or compensation beads.

Compensation controls

Single stain cell controls are recommended for the most accurate compensation. Compensation beads could be used to overcome limited sample availability, but compensation may not be as accurate as with cell controls.



Human PBMCs were stained with the T cell backbone panel. Either single stained cells or BD® CompBeads Compensation Particles Set, as per manufacturer's instructions, were used as compensation controls. **A)** Panel resolution after applying cell-based compensation. **B)** Panel resolution after applying bead-based compensation. Compensation was calculated using the AutoSpill algorithm in FlowJo™ Software.



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tips&tricks

Titer antibodies, stimulus concentration and incubation time to ensure resolution of potentially impacted surface markers and sufficient cytokine production, proliferation and protein phosphorylation.

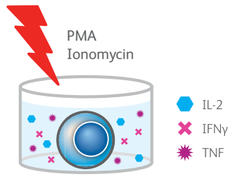
The protocols and reagents for cell activation vary depending on the cytokine and the species of interest.

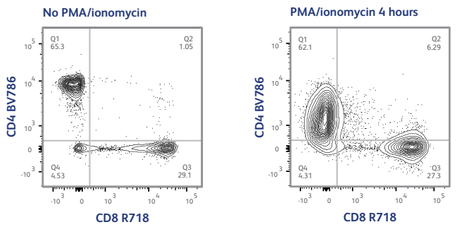
Explore more cell activation protocols
Click to learn more

Cell activation

Immune cell activation is often required to induce cytokine production, proliferation and/or protein phosphorylation.

Treatment with stimuli such as phorbol myristate acetate (PMA) and ionomycin may induce surface receptor downregulation and/or internalization, resulting in potential loss of resolution.





Magnetically enriched human T cells were cultured at 37 °C for 4 hours with or without PMA/ionomycin, prior to staining with the backbone panel. CD4 downregulation and/or internalization upon PMA/ionomycin treatment may impact the ability to resolve CD4+ cells.

tips&tricks

Perform stain with FVS before fixation in protein-free buffer (e.g., 1X PBS) to avoid dye sequestration and suboptimal stain.

Wash cells stained with FVS with buffer with protein in it (FBS or BSA) to eliminate unbound dye and reduce background.

FVS titration is recommended for specific cell types and/or applications.

If nuclear dyes are used for DNA content analysis, rather than dead cell exclusion, the spillover spread impact must be taken in account as this could impact the backbone and/or the drop-in fluorochromes.

Learn more about nucleic acid stains

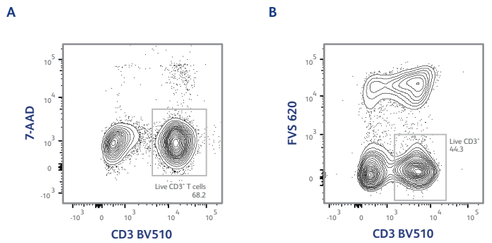
Learn more about FVS

Viability stain

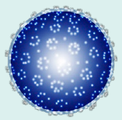
Viability dyes enable exclusion of dead cells and staining artifacts thereof. This is particularly important when analyzing samples with high numbers of dead cells, such as activated or tissue-derived cells. The choice between two main categories of viability dyes, nucleic acid dyes and fixable viability stains (FVS), depends on the status of the cells (fresh or fixed) and experimental workflow.

Viability dye family	Viability dye examples	Cell status	Assay type	Workflow
Nucleic acid stains	7-AAD, DAPI, PI	Fresh, unfixed cells	No wash required	Surface marker stain
Fixable viability stains	FVS450 FVS510 FVS575V FVS620 FVS780	Fixed or unfixed cells	Wash required	Surface and/or intracellular

The BD Horizon™ Human T Cell Backbone Panel is compatible with both families of viability dyes. Since dead cells are excluded, the spillover spread of these dyes into the backbone or drop-in fluorochromes is irrelevant.



Representative data from two different experiments. **A)** Frozen PBMCs were thawed and stained with the backbone panel together with the viability dye 7-AAD. **B)** PBMCs treated with PMA/ionomycin at 37 °C for 4 hours were stained with the fixable viability stain FVS620 prior to fixation and permeabilization.



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tips&tricks

Let cells incubate or culture with the stimulus for at least an hour before adding the protein transport inhibitor.

Be aware of cell toxicity upon prolonged exposure to protein transport inhibitors (>18 hours).

If prolonged incubation time is required, use the less toxic BD GolgiPlug™ Inhibitor.

When investigating multiple cytokines, cells can be incubated with both BD GolgiStop™ and BD GolgiPlug™ Inhibitors for optimal resolution of each cytokine.

Learn more about intracellular flow cytometry

Intracellular stain

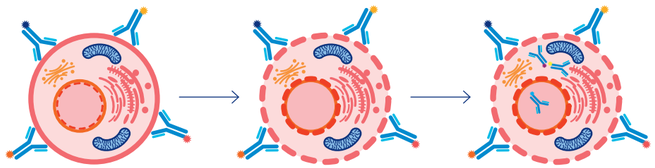
Different fixation and permeabilization buffers may be required for detection of intracellular proteins depending on their cellular localization, e.g., cytoplasmic or nuclear.

Target	Localization	Examples of Fixation/Permeabilization buffer	Composition
Cytokines, cytoplasmic proteins	Cytoplasmic	BD Cytofix/Cytomerm™ Fixation/Permeabilization Kit	PFA/Saponin
Transcription factors	Nuclear	BD Pharmingen™ Transcription Factor Buffer Set	PFA/Saponin
Phosphorylated proteins	Cytoplasmic/nuclear	BD Cytofix™ Fixation Buffer and BD Phosflow™ Perm Buffer III	PFA/Methanol

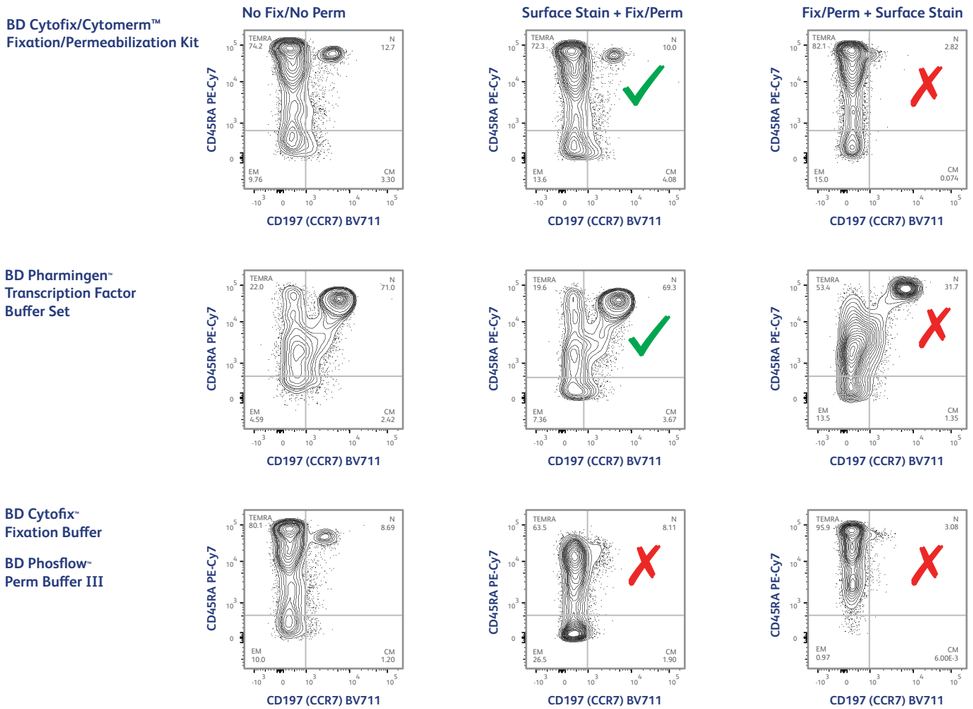
Brightness and spillover of the fluorochromes used in the backbone panel are minimally impacted by BD Cytofix/Cytomerm™ Fixation/Permeabilization Kit and BD Pharmingen™ Transcription Factor Buffer Set. Use of the BD Cytofix™ Fixation Buffer and BD Phosflow™ Perm Buffer III system after surface staining is not recommended due to its known harmful effects on fluorochromes such as PE, PE-Cy7 and BV711.

CD197 epitope integrity is impacted by all three buffer systems tested thus preventing optimal antibody binding when staining is performed after fixation and permeabilization.

A three-step staining protocol is therefore recommended for the use of the BD Horizon™ Human T Cell Backbone Panel when using any of the tested buffer systems.



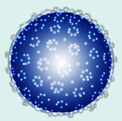
1. Surface staining — 2. Fixation and permeabilization — 3. Intracellular staining



Impact of fixation and permeabilization buffer systems on backbone performance. Representative data from different donors and experiments. Unfixed PBMCs were stained with the BD Horizon™ HumanT Cell Backbone Panel as a reference.

Disclaimers

The impact of representative fixation and permeabilization buffers on the integrity of the backbone panel surface antigens and fluorochromes has been tested on fresh human PBMCs. Further protocol optimization is strongly recommended when using different samples, buffer systems and/or when adding new surface markers.



Learn more about the BD Horizon™ Human T Cell Backbone Panel

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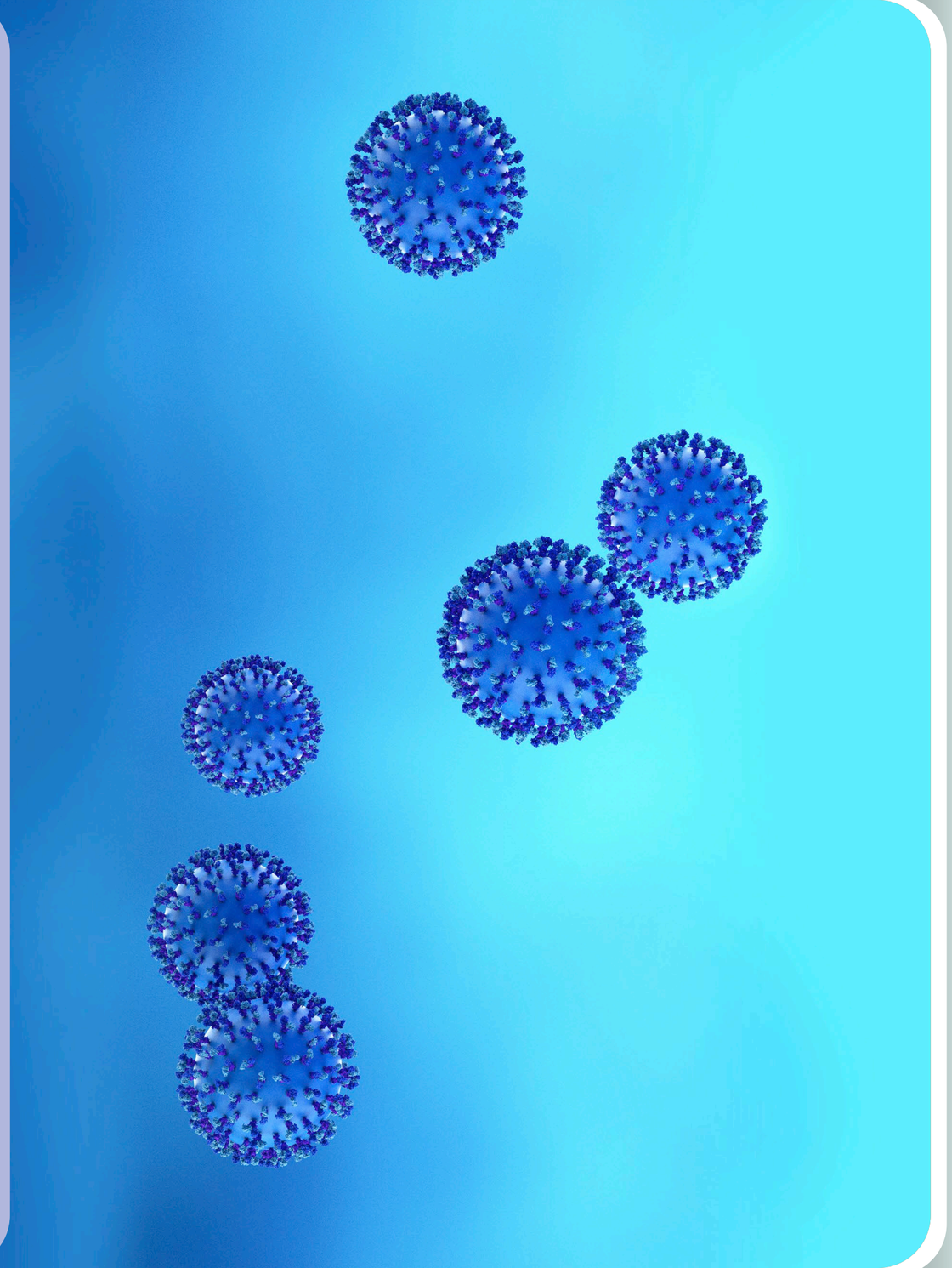
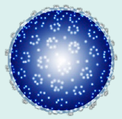


The BD Horizon™ Human T Cell Backbone Panel contains surface markers commonly used to assess the maturation of $CD4^+$ and $CD8^+$ T cells. The unique and strategic design of this backbone panel facilitates the addition of up to five markers to further deepen T cell characterization with minimal panel design effort.

The flexibility of the BD Horizon™ HumanT Cell Panel is demonstrated here by the ability to add a variety of drop-in markers, either surface or intracellular, to study different T cells subsets and within different applications, including:

- $CD4^+$ T helper cell subsets
- T cell maturation
- Polyfunctional T cells
- Regulatory T cells
- T cell activation/exhaustion

The BD Horizon™ HumanT Cell Backbone Panel may also be compatible with the use of PE- or APC-conjugated streptavidin, commonly used for the detection of biotinylated antibodies against chimeric antigen receptors (CARs) or biotinylated MHC multimers for the detection of antigen-specific T cells.



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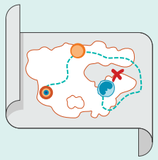

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CD4+ T helper cells subsets

The BD Horizon™ HumanT Cell Backbone Panel was complemented with CD194 (CCR4), CD196 (CCR6), CD183 (CXCR3) and CCR10 drop-ins to identify putative Th1, Th2, Th17 and Th22 subsets of CD4+ T cells. The BD Pharmingen™ 7-AAD Viability Dye was also used to exclude dead cells.

Backbone panel

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD3	UCHT1	BV510	5	568263
Violet	CD4	SK3	BV786	5	
Red	CD8	RPA-T8	R718	5	
Blue or Yellow-green	CD45RA	HI100	PE-Cy7	5	
Violet	CD197 (CCR7)	2-L-1A	BV711	5	
	BD Horizon™ Brilliant Stain Buffer			50	

CD4+ T helper cell subset panel drop-ins

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD194 (CCR4)	1G1	BV421	5	562579
Blue	CD196 (CCR6)	11A9	BB515	5	564479
Blue or Yellow-green	CD183 (CXCR3)	1C6/CXCR3	PE	20	557185
Red	CCR10	1B5	APC	5 µL* 0.06 ug/test	564771
Blue	Live/Dead	N/A	7-AAD	20	559925

* Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

Experimental details

Sample type tested	PBMCs
Protocols	PBMC isolation Surface marker staining
Tested on	3-laser 12-color BD FACSLyric™ Flow Cytometer 4-laser BD FACSymphony™ A1 Flow Cytometer
Companion products	BD Pharmingen™ Human Fc Block™ Reagent

Disclaimers

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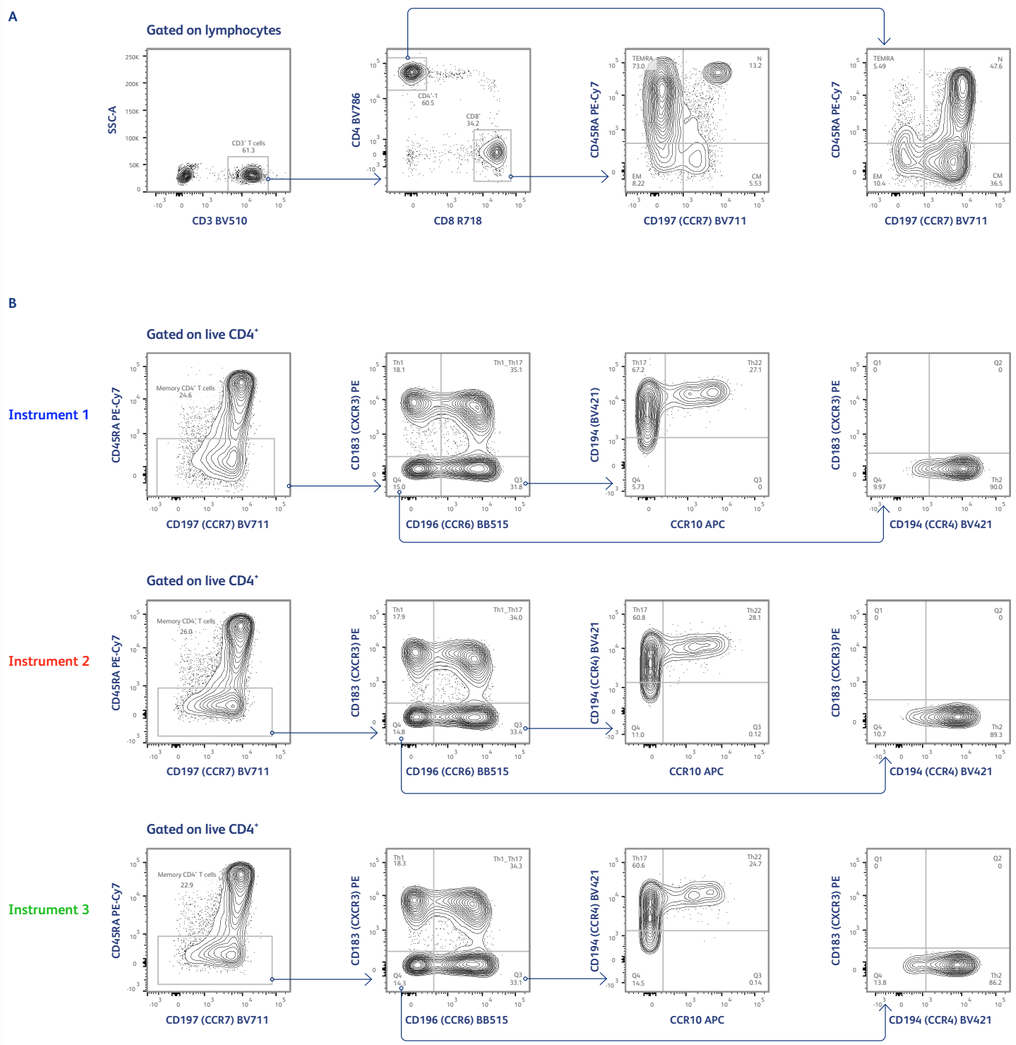
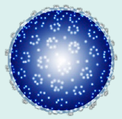


PBMCs from N=2 healthy donors were stained with the BD Horizon™ Human T Cell Backbone Panel alone or together with the CD4+ T helper cell subset drop-ins. Pre-stain at 37 °C for 10 minutes was performed for all the chemokine receptors. **A)** Resolution of T cell subsets using the BD Horizon™ Human T Cell Backbone panel alone. **B)** The addition of the CD4 T helper cell subset subset drop-ins enabled clear resolution of putative Th1, Th2, Th17 and Th1/Th17 subsets within live memory CD4+ cells, based on conventional gating strategy. The same sample was run on three 3-laser 12-color BD FACSLyric™ Flow Cytometers with similar performance. Data were analyzed using FlowJo™ v10.8.1 Software. Gates were drawn based on fluorescence minus one (FMO) controls.



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T cell maturation

The BD Horizon™ Human T Cell Backbone Panel was complemented with mouse anti-human CD95 drop-in to further identify stem cell memory T cells. The addition of early and late differentiation markers CD127, CD27, CD28 and KLRG1 further enabled a deeper assessment of T cell maturation. The BD Pharmingen™ 7-AAD Viability Dye was also used to exclude dead cells.

Backbone panel

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD3	UCHT1	BV510	5	568263
Violet	CD4	SK3	BV786	5	
Red	CD8	RPA-T8	R718	5	
Blue or Yellow-green	CD45RA	HI100	PE-Cy7	5	
Violet	CD197 (CCR7)	2-L-1A	BV711	5	
	BD Horizon™ Brilliant Stain Buffer			50	

T cell maturation panel drop-ins

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
UV	CD28	CD28.2	BUV395	1.25	740308
Violet	KLRG1	Z7-205.rMAb	BV421	5	568268
Blue	CD127	HIL-7R-M21	BB515	5	564423
Blue or yellow-green	CD95	DX2	PE	20	555674
Red	CD27	M-T271	APC	20	558664
Blue	Live/Dead	N/A	7-AAD	20	559925

Experimental details

Sample type tested	PBMCs
Protocols	PBMC isolation Surface marker staining
Tested on	5-laser BD LSRFortessa™ X-20 Flow Cytometer 5-laser BD FACSymphony™ A5 Flow Cytometer
Companion products	N/A

Disclaimers
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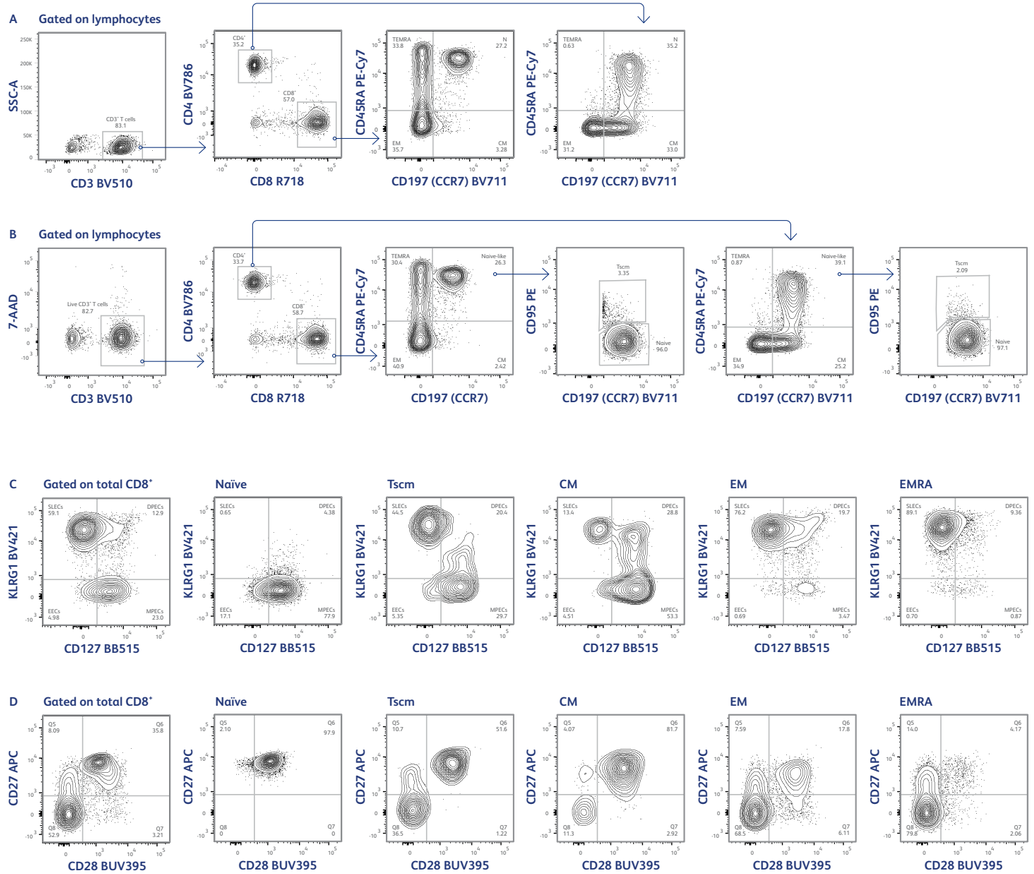
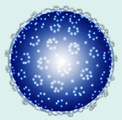


Human PBMCs from N=2 healthy donors were isolated and stained with the BD Horizon™ Human T Cell Backbone Panel alone or together with the indicated drop-ins. **A)** Representative resolution of the CD4+ and CD8+ T cell subsets using the backbone panel. **B)** The addition of five drop-ins did not impact the resolution of the T cell subsets defined by the backbone panel. The addition of CD95 enabled the separation of CD4+ and CD8+ naïve-like cells into CD95- naïve and CD95+ stem cell memory (Tscm) subsets. **C-D)** A deeper characterization of CD8+ cells and subsets thereof was further enabled by the additional drop-ins KLRG1, CD127, CD27 and CD28. Samples were acquired on a 5-laser BD FACSymphony™ A5 Flow Cytometer and data were analyzed using FlowJo™ v10.8.1 Software. Gates were drawn based on FMO controls.



Learn more about the BD Horizon™ Human T Cell Backbone Panel

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Polyfunctional T cells

The BD Horizon™ Human T Cell Backbone Panel was complemented with BD Horizon™ Violet Proliferation Dye (VPD) 450 and antibodies against the pro-inflammatory cytokines IL-2, IFN-γ and TNF. The BD Horizon™ Fixable Viability Stain (FVS) 620 was also used to exclude dead cells. The use of the backbone alone allows for identification of changes in the maturation status of T cells upon activation. The addition of the drop-ins further enables assessment of cell function and response to activation measured as proliferation rate and cytokine production.

Backbone panel

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD3	UCHT1	BV510	5	568263
Violet	CD4	SK3	BV786	5	
Red	CD8	RPA-T8	R718	5	
Blue or Yellow-green	CD45RA	HI100	PE-Cy7	5	
Violet	CD197 (CCR7)	2-L-1A	BV711	5	
	BD Horizon™ Brilliant Stain Buffer			50	

Polyfunctional T cell panel drop-ins

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	Violet Proliferation Dye 450	N/A	VPD 450	1:1000*	562158
Blue	IFN-γ	B27	FITC	20 µL	552887
Yellow-green	IL-2	MQ1-17H12	PE	5 µL	559334
Red	TNF	MAb11	APC	20 µL	551384
Blue	Live/Dead	N/A	FVS620	5*	564996

*From the stock solution

Experimental details

Sample type tested	Resting and activated PBMCs
Protocols	PBMC isolation T cell activation Surface marker staining Intracellular staining
Tested on	4-laser BD FACSymphony™ A1 Flow Cytometer
Companion products	BD GolgiPlug™ and BD GolgiStop™ Protein Transporter Inhibitors BD Cytofix/Cytomerm™ Fixation/Permeabilization Kit

Disclaimers

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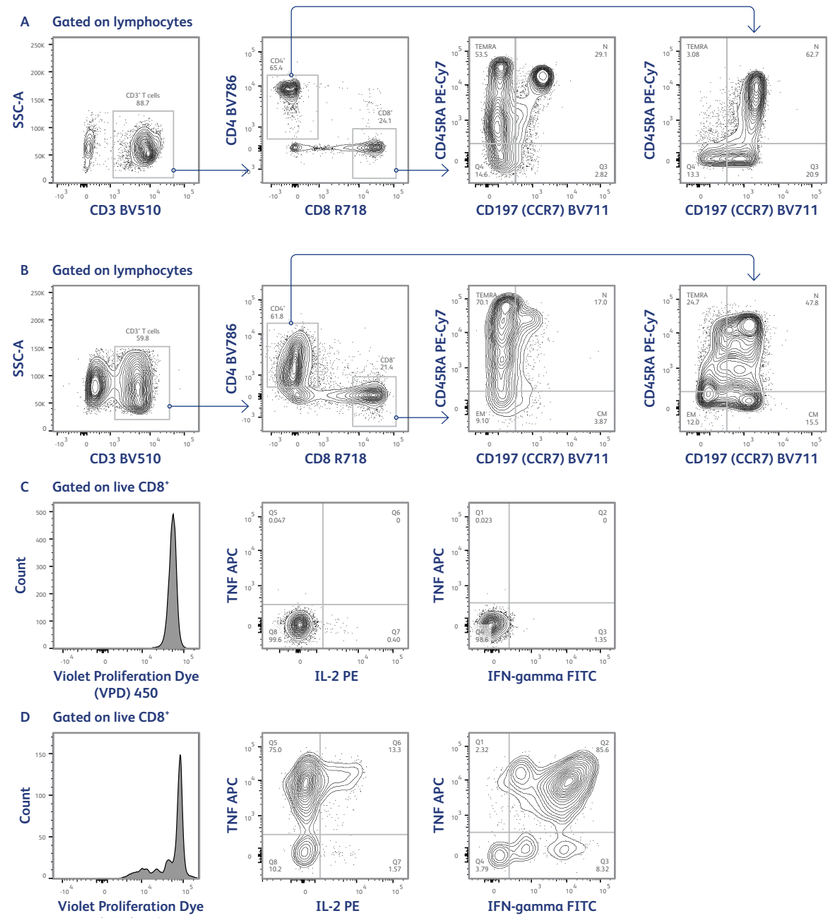
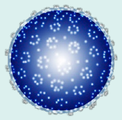


Human PBMCs were derived from N=2 healthy donors. Prior to culture, cells were stained with BD Horizon™ Violet Proliferation Dye 450, as per manufacturer's instructions. Cells were cultured in complete medium in the presence of Dynabeads™ Human T-Activator CD3/CD28 for 3 days. Four hours prior to collection, cells were treated with PMA/ionomycin (50 ng/mL and 10 µg/mL) and BD GolgiPlug™ or BD GolgiStop™ Protein Transporter Inhibitors at 37 °C. Resting cells were cultured for 3 days in complete medium without Dynabeads™ Human T-Activator CD3/CD28 and without further PMA/ionomycin stimulation. Resting cells were used as biological controls. Cells were then collected and stained with the BD Horizon™ Human T Cell Backbone Panel, followed by fixation and permeabilization with BD Cytofix/Cytoperm™ Buffer and cytokine intracellular stain. **A-B)** Representative resolution of T cell subsets using the backbone panel alone. As expected, reduced CD4 signal and increased frequency of terminally differentiated cells was observed in activated cells (**B**), as compared to resting cells (**A**). The addition of the polyfunctional T cell drop-ins and the viability dye FVS620 enabled a deeper characterization of activated cells. Dilution of BD Horizon™ Violet Proliferation Dye 450 indicated proliferation of live CD8+ T cells upon activation (**D**), as compared to resting cells (**C**). Cell activation also induced expression of pro-inflammatory cytokines TNF, IL-2 and IFN-gamma in activated cells (**D**), as compared to resting cells (**C**). Samples were acquired on a 4-laser BD FACSymphony™ A1 Flow Cytometer and data were analyzed using FlowJo™ Software. Gates were drawn based on FMO and biological controls.



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CD4+ regulatory T cells

The BD Horizon™ Human T Cell Backbone Panel was complemented with CD25 and CD127 drop-ins for detection of CD25+CD127low putative CD4+ regulatory T cells (Tregs). Intracellular detection of the transcription factor FoxP3 allowed for a more refined definition of Tregs. A deeper Treg characterization was further achieved by the use of the drop-in CD15s, together with the backbone markers CD45RA and CD197 (CCR7), for the identification of naïve and effector Treg subsets. The fixable viability stain FVS620 was used to exclude dead cells.

Backbone panel

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD3	UCHT1	BV510	5	568263
Violet	CD4	SK3	BV786	5	
Red	CD8	RPA-T8	R718	5	
Blue or Yellow-green	CD45RA	HI100	PE-Cy7	5	
Violet	CD197 (CCR7)	2-L-1A	BV711	5	
BD Horizon™ Brilliant Stain Buffer				50	

CD4+ regulatory T cell panel drop-ins

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD127	HIL-7R-M21	BV421	5	562436
Blue	FoxP3	236A/E7	AF488	20	561181
Yellow-green	CD25	2A3	RY586	5	568124
Red	HLA-DR	G46-6	APC	20	559866
Blue	Live/Dead	N/A	FVS620	5*	564996

*From the stock solution

Experimental details

Sample type tested	PBMCs
Protocols	PBMC isolation Surface marker staining Intracellular staining
Tested on	4-laser BD FACSymphony™ A1 Flow Cytometer
Companion products	BD Pharmingen™ Transcription Factor Buffer Set

Disclaimers

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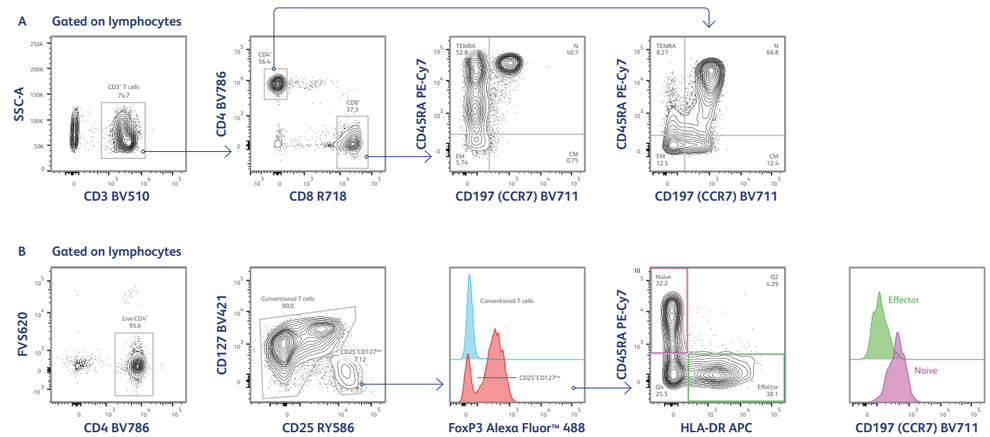
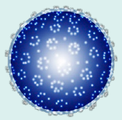


Human PBMCs were isolated from N=3 healthy donors and stained with the BD Horizon™ Human T Cell Backbone Panel together with the surface marker drop-ins CD25, CD127 and CD15s. Cells where then fixed and permeabilized using BD Pharmingen™ Transcription Factor Buffer Set, as per manufacturer's instructions, prior to intracellular FoxP3 stain. **A)** Representative resolution of T cell major subsets using the BD Horizon™ Human T Cell Backbone panel alone on fresh PBMCs. **B)** The addition of the drop-ins, used in combination with the markers present in the backbone panel, enabled the identification of CD25+CD127lowFoxP3+ Tregs. Naïve and effector Tregs could be further characterized based on differential expression of CD45RA, HLA-DR and CD197. Samples were acquired on a 4-laser BD FACSymphony™ A1 Flow Cytometer. Gates were drawn based on FMO controls.



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T cell activation/exhaustion

The BD Horizon™ Human T cell Backbone Panel was complemented with CD279 (PD-1), CD366 (TIM-3) and CD223 (LAG-3) drop-ins. Expression of these inhibitory receptors is upregulated upon T cell activation or exhaustion. Staining with the backbone alone allows for the detection of changes in CD4+ and CD8+ T cell maturation upon activation. Upregulation of the inhibitory receptor drop-ins can be further evaluated upon T cell activation. The combination of backbone and drop-in markers enable an even deeper cell characterization based on the analysis of the inhibitory receptor expression patterns within distinct CD4+ or CD8+ naïve, central memory, effector memory and TEMRA subsets. In this panel, the PE channel was strategically left open for potential detection of biotinylated anti-CAR antibodies or MHC multimers through PE-Streptavidin stain.

Backbone panel

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	CD3	UCHT1	BV510	5	568263
Violet	CD4	SK3	BV786	5	
Red	CD8	RPA-T8	R718	5	
Blue or Yellow-green	CD45RA	HI100	PE-Cy7	5	
Violet	CD197 (CCR7)	2-L-1A	BV711	5	
	BD Horizon™ Brilliant Stain Buffer			50	

T cell activation/exhaustion panel drop-ins

Laser	Marker	Clone	Fluorochrome	µL/test	Catalog Number
Violet	PD-1	EH12.1	BV421	5	562516
Blue	TIM3	7D3	BB515	5	565568
Red	LAG3	T47-530	AF647	5	565716
Blue	Live/Dead	N/A	7-AAD	20	559925

Experimental details

Sample type	PBMCs, cultured and activated T cells
Protocols	PBMC isolation T cell magnetic enrichment T cell activation Surface marker staining
Tested on	3-laser 12-color BD FACSLyric™ Flow Cytometer
Companion products	BD IMag™ Human T Lymphocyte Enrichment Set-DM

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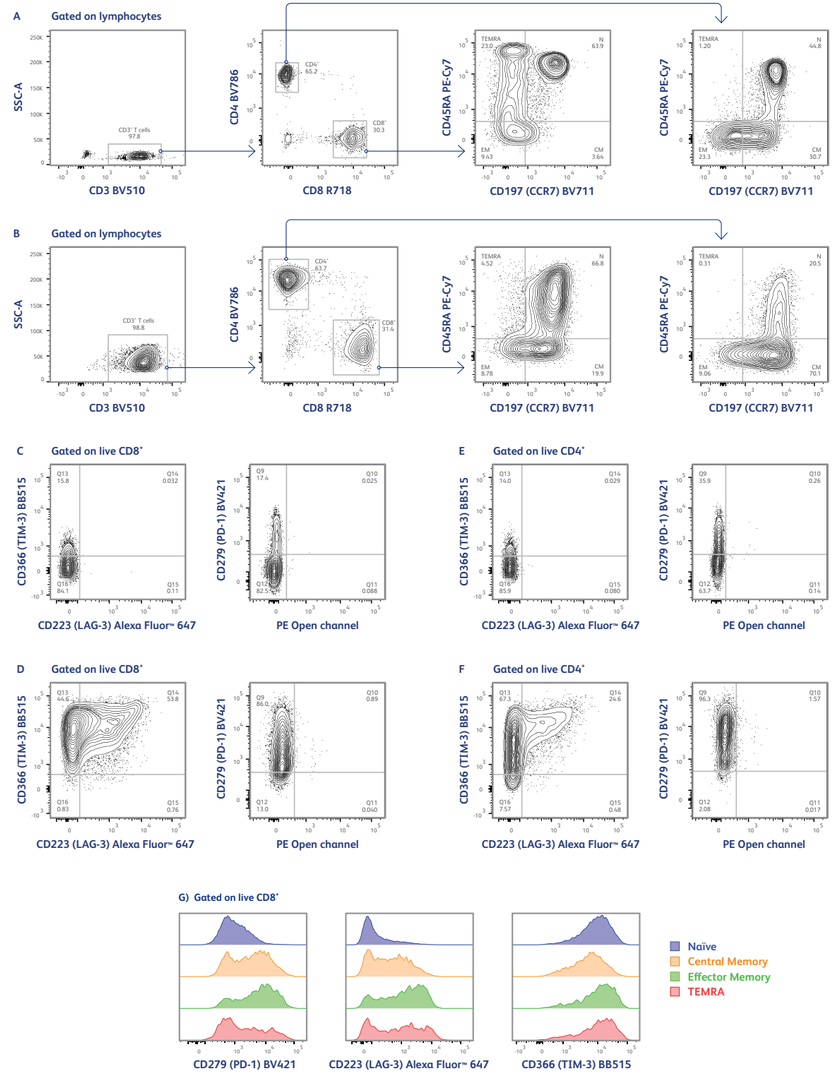

Human T cells were magnetically enriched from N=2 healthy donors using the BD IMag™ Human T Lymphocyte Enrichment Set – DM. Cells were cultured in complete medium in the presence of Dynabeads™ Human T-Activator CD3/CD28 for 2 days. Resting cells were cultured for 2 days in complete medium without Dynabeads™ Human T-Activator CD3/CD28 and used as a biological control. **A-B)** Representative resolution of the T cell subsets using the BD Horizon™ Human T Cell Backbone Panel. As expected, differences in the distribution of CD4+ and CD8+ naïve and memory subsets were observed between resting (**A**) and activated cells (**B**). The addition of the drop-ins and the viability dye 7-AAD further demonstrated upregulation of the inhibitory receptors PD-1, TIM-3 and LAG-3 within live CD8+ activated cells (**D**), as compared to resting cells (**C**). (**E-F**) Similar observations were made for live CD4+ cells. The PE channel was strategically open for potential detection of biotinylated anti-CAR antibodies or MHC multimers through PE-Streptavidin stain. **G)** Assessment of inhibitory receptor expression on distinct subsets of activated CD8+ T cells. Samples were acquired on a 3-laser, 12-color BD FACLyric™ Flow Cytometer and data were analyzed using FlowJo™ Software. Gates were drawn based on FMO and biological controls.

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BD Cytofix/Cytomerm™ Fixation/Permeabilization Kit	BD Horizon Brilliant™ Violet 786 (BV786) Reagents
BD FACSLyric™ Flow Cytometer	BD Horizon™ Human T Cell Backbone Panel
BD FACSymphony™ A1 Cell Analyzer	BD Horizon™ Red 718 Reagents
BD FACSymphony™ A5 SE Cell Analyzer	BD Horizon™ Violet Proliferation Dye 450
BD GolgiPlug™ Protein Transporter Inhibitors	BD IMag™ Human T Lymphocyte Enrichment Set – DM
BD GolgiStop™ Protein Transporter Inhibitors	BD LSRFortessa™ X-20 Cell Analyzer
BD Horizon Brilliant™ Blue Reagents	BD Pharmingen™ Transcription Factor Buffer Set
BD Horizon™ Brilliant Stain Buffer	BD Phosflow™ Perm Buffer III
BD Horizon Brilliant™ Ultraviolet (BUV395) Reagents	FlowJo™ Software

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