Cancer Research *is* Complicated

Connect the Dots with BioLegend's

Immuno-Oncology Research Tools

BioLegend is ISO 9001:2008 and ISO 13485:2003 Certified

BioLegend®

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Tel: 858.768.5800
biolegend.com

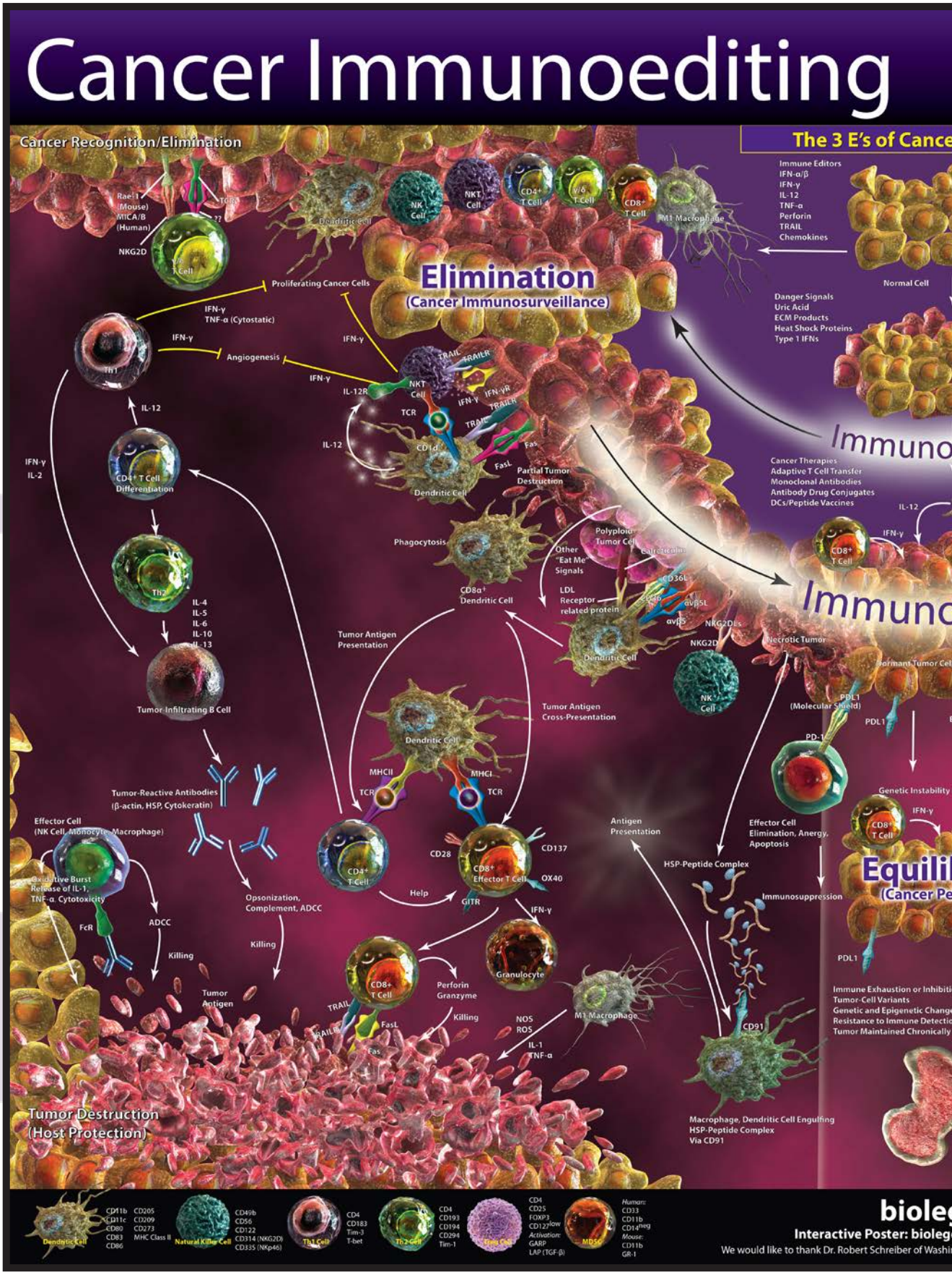
02-0013-00

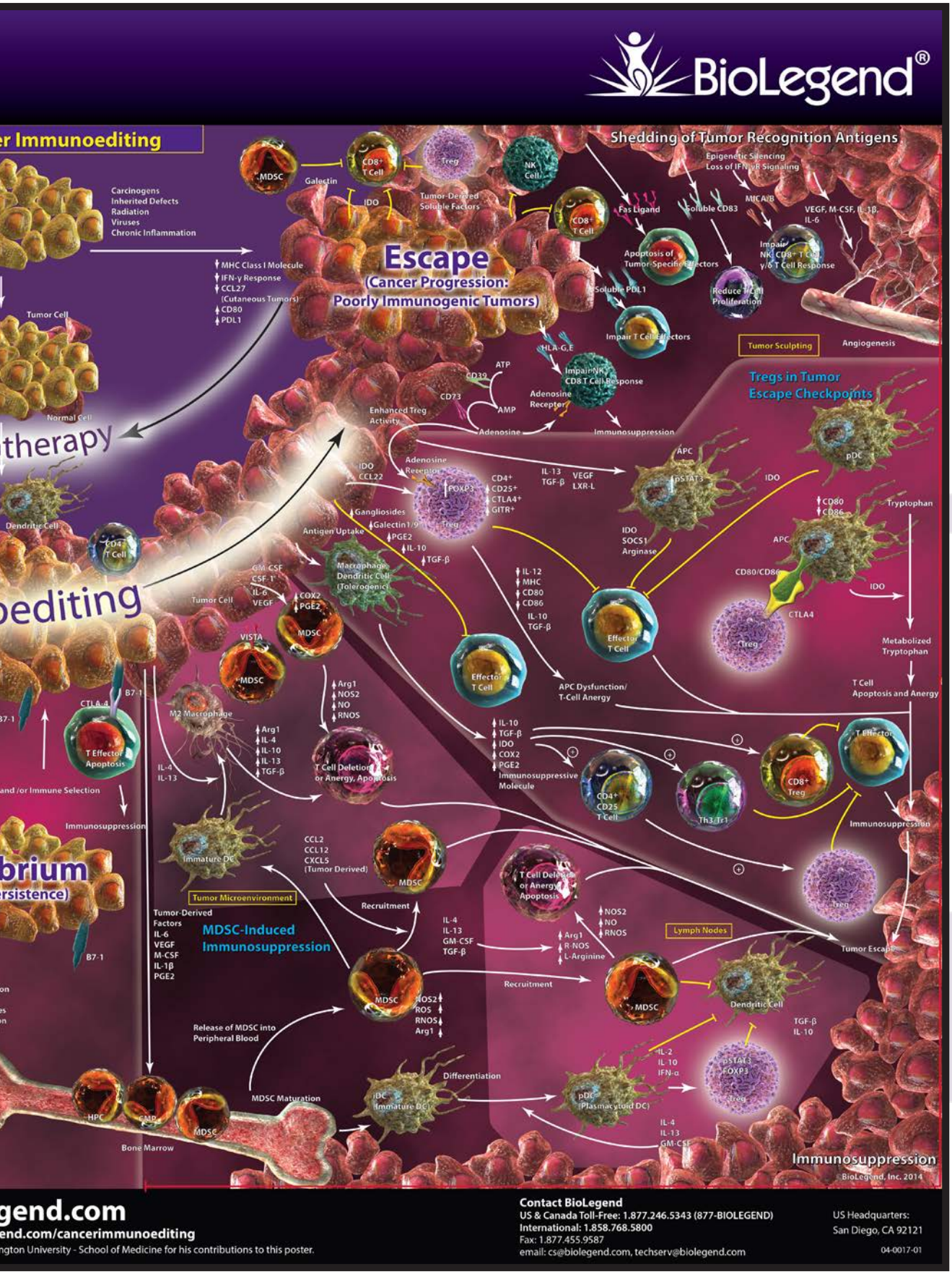
World-Class Quality | Superior Customer Support | Outstanding Value

Contents

In recent years, the involvement of immune cells in controlling cancers has come to the forefront of therapy and research. It is now evident that immune cells are central to the three "E's" of cancer immunoediting: Elimination, Equilibrium, and Escape, as tumors devise a diverse set of mechanisms to overcome immune control. One critical mechanism is the expression of immune checkpoint ligands on tumors that engage checkpoint receptors on immune cells, resulting in immune inactivation and tumor escape. Targeting this pathway with blocking antibodies has shown real promise, but continued research is essential. BioLegend provides an extensive array of research tools for immuno-oncology research and continues our commitment as a preferred supplier to the Parker Institute for Cancer Immunotherapy.

Cancer Immunoediting pathways




BioLegend®
Immunoediting
Shedding of Tumor Recognition Antigens
Epigenetic Silencing
Loss of IFN-γR Signaling
Carcinogens
Inherited Defects
Radiation
Viruses
Chronic Inflammation
MDSC
Galectin
CD8+ T Cell
Treg
NK Cell
IDO
Tumor-Derived Soluble Factors
Fas Ligand
Soluble CD83
MICA/B
VEGF, M-CSF, IL-1β, IL-6
Escape
(Cancer Progression:
Poorly Immunogenic Tumors)
MHC Class I Molecule
IFN-γ Response
CCL27
(Cutaneous Tumors)
CD80
PDL1
Tumor Cell
Normal Cell
Apoptosis of Tumor-Specific Effectors
Impair NK, CD8+ T Cell, γ/δ T Cell Response
Soluble PDL1
Reduce T Cell Proliferation
Impair T Cell Effectors
Tumor Sculpting
Angiogenesis
HLA-G,E
ATP
CD39
CD73
AMP
Adenosine Receptor
Impair NK, CD8 T Cell Response
Tregs in Tumor
Escape Checkpoints
Enhanced Treg Activity
Adenosine
Immunosuppression
therapy
APC
pDC
IDO
CCL22
Adenosine Receptor
FOXP3
CD4+ CD25+ CTLA4+ GITR+
IL-13
TGF-β
VEGF
LXR-L
pSTAT3
Tryptophan
CD80
CD86
Gangliosides
Galectin1/9
PGE2
IL-10
TGF-β
Antigen Uptake
IDO
SOCS1
Arginase
Dendritic Cell
CD4+ T Cell
GM-CSF
CSF-1
IL-6
VEGF
Macrophage
Dendritic Cell
(Tolerogenic)
IL-12
MHC
CD80
CD86
IL-10
TGF-β
CD80/CD86
CTLA4
Treg
Effector T Cell
editing
Tumor Cell
COX2
PGE2
MDSC
VISTA
Metabolized Tryptophan
T Cell Apoptosis and Anergy
APC Dysfunction/
T-Cell Anergy
B7-1
CTLA-4
M2 Macrophage
Arg1
NOS2
NO
RNOS
T Effector Apoptosis
Arg1
IL-4
IL-10
IL-13
TGF-β
T Cell Deletion or Anergy, Apoptosis
IL-4
IL-13
IL-10
TGF-β
IDO
COX2
PGE2
Immunosuppressive Molecule
CD4+ CD25 T Cell
Th3 Tr1
CD8+ Treg
T Effector
and /or Immune Selection
Immunosuppression
brium
rsistence)
Immature DC
CCL2
CCL12
CXCL5
(Tumor Derived)
MDSC
T Cell Deletion or Anergy, Apoptosis
Tumor Microenvironment
Recruitment
Tumor-Derived Factors
IL-6
VEGF
M-CSF
IL-1β
PGE2
MDSC-Induced Immunosuppression
IL-4
IL-13
GM-CSF
TGF-β
Arg1
R-NOS
L-Arginine
NOS2
NO
RNOS
Lymph Nodes
Tumor Escape
Recruitment
MDSC
MDSC
Dendritic Cell
Release of MDSC into Peripheral Blood
NOS2
ROS
RNOS
Arg1
TGF-β
IL-10
Differentiation
IL-2
IL-10
IFN-α
pSTAT3
FOXP3
Treg
MDSC Maturation
iDC (Immature DC)
pDC (Plasmacytoid DC)
HPC
CMP
MDSC
Bone Marrow
IL-4
IL-13
GM-CSF
Immunosuppression
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ngton University - School of Medicine for his contributions to this poster.
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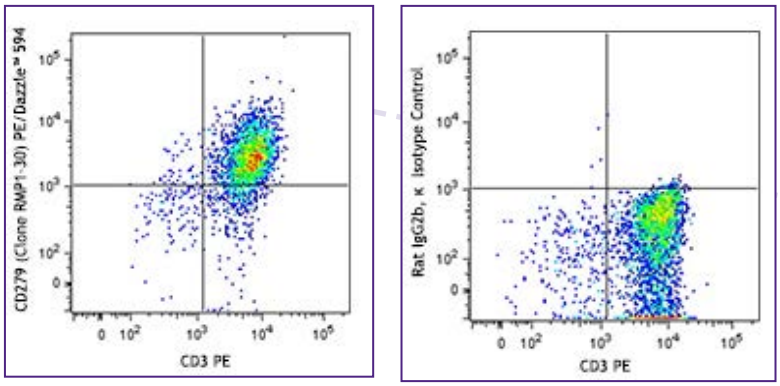
San Diego, CA 92121
04-0017-01

Flow Cytometry Antibodies

Accurate immune status monitoring of cancer patients is critical during immunotherapy as well as other oncology therapies. BioLegend provides 12,000 purified and fluorophore conjugated antibodies for flow cytometry with extensive quality testing for flow cytometry. Our selection of fluorophores includes the family of Brilliant Violet™ fluorophores, including BV421™, and our novel fluorophores PE/Dazzle™ 594 and APC/Fire™ 750. Our antibody clones are often provided with a large selection of fluorophore options, allowing for flexibility in developing the best flow cytometry panels.

Learn more about our flow cytometry tools at:
biolegend.com/immunobiology

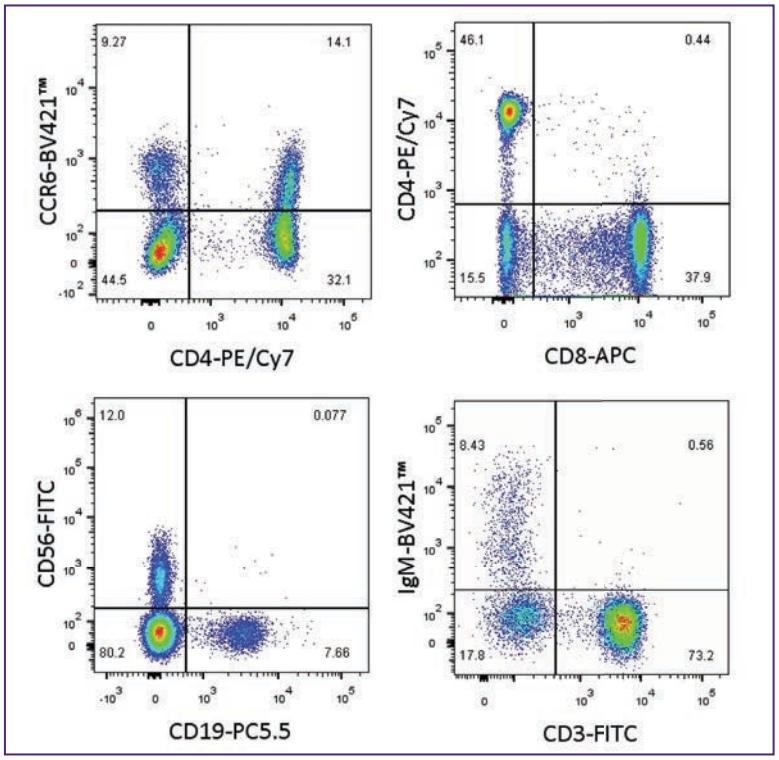


Con A (three-days) activated C57BL/6 splenocytes were stained with CD3 PE and CD279 (clone RMP1-30) PE/Dazzle™ 594 (left) or rat IgG2b, κ PE/Dazzle™ 594 isotype control (right).

Veri-Cells™

Accurate controls are essential for long-term studies and reproducibility across research sites. Reference laboratories, clinical research organizations, and multi-center clinical trials, among other institutions, need reliable controls to monitor assay performance and variability for longitudinal studies. Our unique lyophilized control cell products: Veri-Cells™ PBMC and CD4-Low PBMC are prepared from human peripheral blood, available as controls to monitor normal and low levels of $CD4^+$ cells. Veri-Cells™ Leukocyte is an excellent human immunophenotyping control, as it includes all leukocyte subsets (lymphocytes, monocytes and granulocytes).

Learn more about Veri-Cells™ at:
biolegend.com/veri-cells



Cell surface staining of various markers on Veri-Cells™ PBMCs.

GoInVivo™

For researchers interested in no-hassle bulk quantities of biofunctional antibodies for mouse injection or human *ex vivo* studies, GoInVivo™ provides the best solution at exceptional prices. GoInVivo™ products focus on targeting immune checkpoints and other important molecules. Tumor cells can express high levels of these checkpoint receptors, shutting down immune responses and taking advantage of the lack of inflammation.

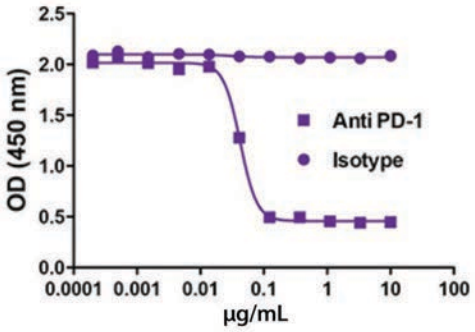
Learn more about these interactions with our Immune Checkpoints Webpage at: biolegend.com/immune_checkpoints

GoInVivo™ features:

- Flow cytometry quality testing
- Super low endotoxin, ≤ 1.0 EU/mg
- Low aggregation, < 3%
- Pathogen-free
- Bulk sizing (5 mg, 25 mg, 50 mg, 100 mg, 500 mg, 1 g sizes)
- Concentration between 1 mg/mL and 3 mg/mL
- Custom sizes available
- Sodium azide and preservative-free

Learn more about GoInVivo products at: biolegend.com/goinvivo

Anti-human PD-1 (clone EH12.2H7)



Anti-human PD-1 inhibits the binding of PD-L1. Immobilized PD-1-Fc was pre-incubated with increasing concentrations of anti-human PD-1 (clone EH12.2H7, purple squares) or isotype control (clone MOPC-21, purple circles), followed by incubation with a fixed concentration of PD-L1-Fc (1 µg/mL). Clone EH12.2H7 inhibits PD-1/PD-L1 interaction in a dose dependent manner.

Flex-T™

Soluble MHC molecules are commonly used to identify antigen-specific T cells that react to cancers. Flex-T™ is BioLegend's technology to study antigen-specific T cells through their TCRs. It has the unique property of allowing the loading of peptides of interest into the binding site of the MHC groove, by using ultraviolet (UV) light labile, exchangeable peptides.

Flex-T™ technology features:

- Flexible peptide loading
- Two color combination capability
- High throughput screening
- High specificity
- Ease of use
- Affordability

Learn more about Flex-T™ at: biolegend.com/flex-t

Peptide of interest mixed with labile peptide-loaded Flex-T™ monomers

UV light degrades labile peptide, allowing for substitution by peptide of interest

Mix peptide-exchanged monomers with fluorophore-Streptavidin to produce tetramers

Identify Antigen-specific CD8⁺ T Cell

Flex-T™ is made of MHC monomers loaded with a peptide that can be degraded by the use of a UV light source. This allows for a peptide exchange when the UV irradiation is done in the presence of the peptide of interest (which is not UV-labile). This flexibility permits the screening of virtually any peptide of interest with enough affinity for the MHC allele that it is loaded onto.

MojoSort™ Magnetic Cell Separation System

MojoSort™ is BioLegend's magnetic cell separation system for the isolation and purification of cells from heterogeneous populations. MojoSort™ offers outstanding performance at an excellent price.

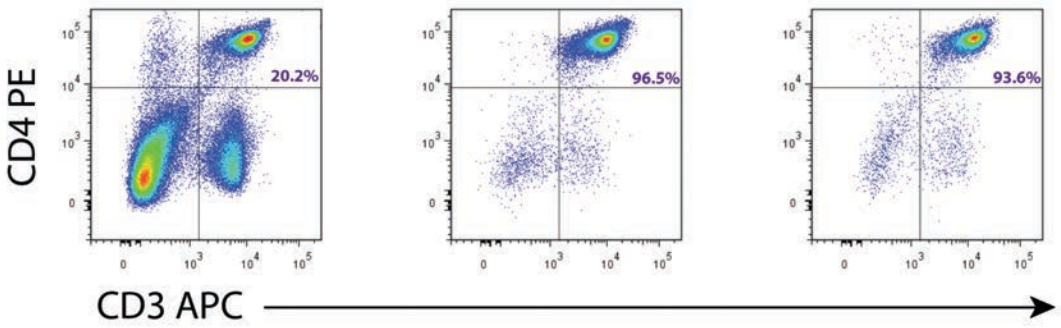
Separation of cells can occur via "Positive Selection" or "Negative Selection" (also known as "Negative Depletion"). This depends on whether the bead-bound antibodies directly target your cells of interest (positive selection) or target unwanted cells (negative selection). Either way, both separated fractions of cells can be used for downstream applications.

MojoSort™ Nanobeads are magnetic particles directly conjugated to antibodies (positive selection) or Streptavidin. MojoSort™ Isolation Kits typically contain a biotin-antibody cocktail and Streptavidin Nanobeads, intended to isolate an untouched cell population.

To use these products, you also need a separation buffer and a magnetic separation system.

We recommend:

- MojoSort™ Buffer (Cat. No. 480017)
- MojoSort™ Magnet (Cat. No. 480019)



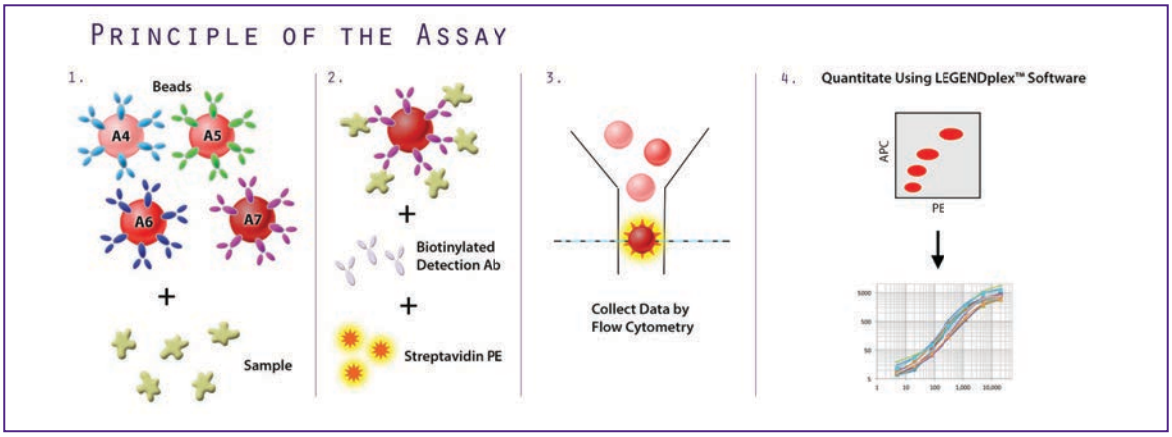
A single cell suspension from pooled C57BL/6 mouse spleen and lymph nodes was prepared to isolate CD4+ T cells using the MojoSort™ Mouse CD4 T Cell Isolation Kit. Cells were stained with PE anti-mouse CD4 (clone RM4-4), APC anti-mouse CD3ε (145-2C11), and 7-AAD. Dead cells were excluded from the analysis.

Learn more about MojoSort™ at: biolegend.com/mojosort

LEGENDplex™

In cancer states, measuring soluble biomarkers is important in understanding immune states, tumor status, or tumor environments. BioLegend's LEGENDplex™ bead-based immunoassays quantify multiple soluble analytes simultaneously in biological samples using a flow cytometer. LEGENDplex™ kits are provided with predefined panels, ranging from 3 to 13 specificities, or customers can mix and match any subset within each predefined panel using our Mix and Match system.

Learn more about LEGENDplex™ at: biolegend.com/legendplex



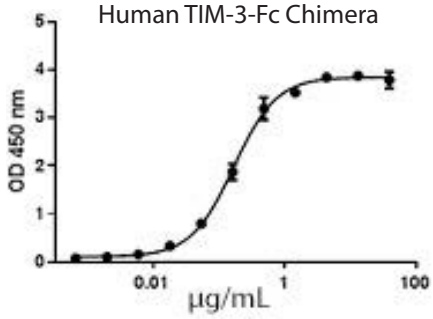
Recombinant Proteins

BioLegend provides an extensive selection of bioactive recombinant proteins for cancer research, including cytokines, growth factors, chemokines, and enzymes, such as matrix metalloproteinases. Products are manufactured by BioLegend and quality tested for bioactivity comparable to or better than leading competitors.

Recombinant Protein features:

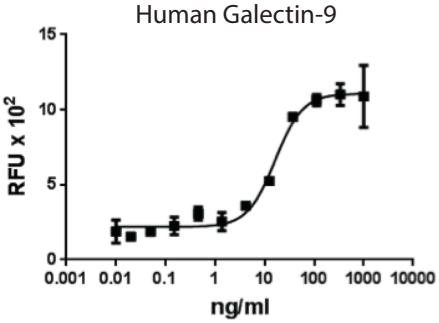
- Proteins for human, mouse, and rat
- Over 95% purity
- In-house validation through bioassays relevant to the function of the protein
- Endotoxin tested to ensure compatibility with biological systems
- Constructed with mammalian, bacterial, or insect protein expression system
- *In-vivo* and *in-vitro* functional assay applications

Human TIM-3-Fc Chimera



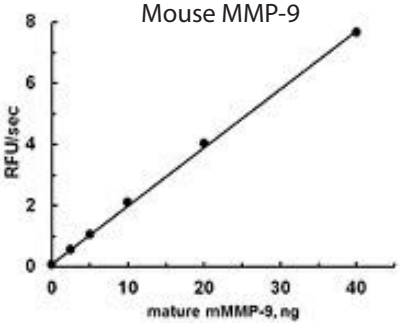
Recombinant human TIM-3 binds immobilized recombinant human Galectin-9 with EC50 of 0.15 - 0.6 µg/mL in a functional ELISA.

Human Galectin-9



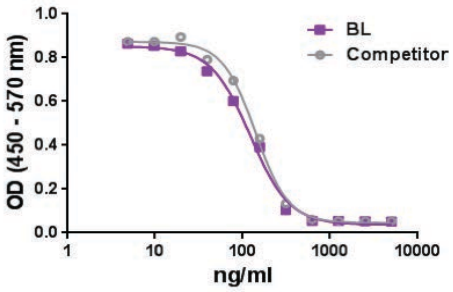
Immobilized recombinant human Galectin-9 induces adhesion of Jurkat cells in a dose dependent manner.

Mouse MMP-9



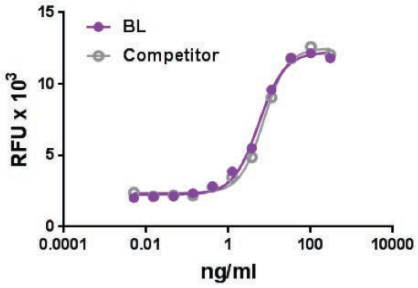
The activity of Mouse MMP-9 was measured in the presence of a fluorogenic substrate.

Human CTLA-4



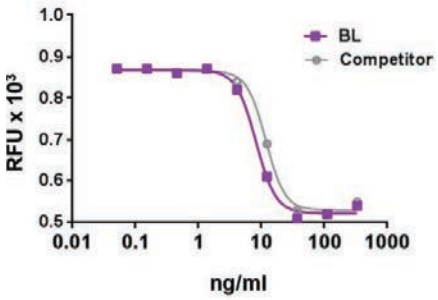
Human CTLA4 inhibition of IL-2 induced by B7.1 (500 ng/ml) in Jurkat acute T cell leukemia cells. BioLegend's protein was compared side-by-side to a competitor's equivalent product.

Mouse M-CSF



Recombinant mouse M-CSF induces proliferation of M-NFS-60 cells in a dose dependent manner. BioLegend's protein was compared side-by-side to a competitor's equivalent product.

Human TRAIL

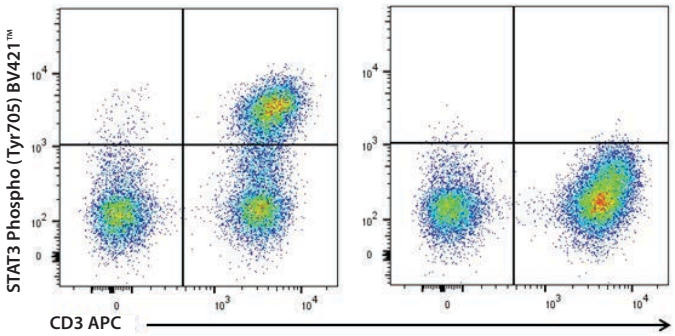


Recombinant human TRAIL (TNFSF10) induces cytotoxicity in mouse L929 cell line in a dose dependent manner. BioLegend's protein was compared side-by-side to the leading competitor's equivalent product.

Learn more about our Recombinant Proteins at: biolegend.com/recombinant_proteins

Phospho-specific Antibodies

Phosphorylation, a mechanism by which phosphoryl groups are added to proteins, is important in a number of cancer-related processes. It is involved in the control of proliferation, oncogenic kinase signaling, transcriptional regulation, p53 tumor suppressing activity, and many other processes. BioLegend offers a wide array of phospho protein-specific antibody products for use in flow cytometry, western blot, and microscopy applications. Whenever possible, the phospho protein-specific antibodies we offer are optimized to work for multiple applications, allowing for clone and product consistency across different experimental systems.



Human whole blood stimulated with IL-6 for 15 minutes (left panel) or unstimulated (right panel), were treated with RBC Lysis/Fixation Solution (10X), permeabilized with True-Phos™ Perm Buffer, and stained with CD3 APC and STAT3 Phospho (Tyr705) Brilliant Violet 421™.

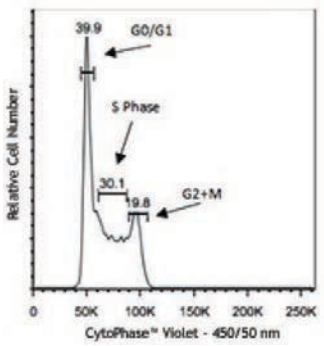
Learn more about protein phosphorylation and view our complete selection of phospho-specific antibodies at: biolegend.com/phospho

Nucleic Acid Stains and Live/Dead Probes

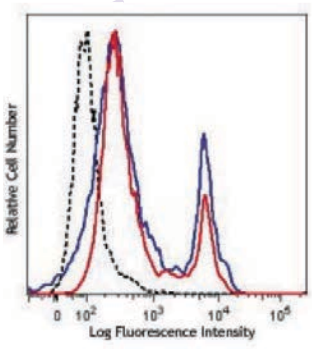
Changes in cell health, apoptosis, cell cycle and proliferation are central indicators of disease progression and therapeutic efficacy in immuno-oncology. For the simple determination of live versus dead status, often the increasing permeancy of the cell membrane is exploited. Impermeant nucleic acid stains like the Helix NP™ family of dyes, DRAQ7™ and Propidium Iodide are used for live/dead status since they gain entrance to a cell with a compromised membrane to stain the nucleus. In flow cytometry, some permeant nucleic acid stains like DRAQ5™ and CytoPhase™ Violet can be used to assess the cell cycle status of live cells. Nucleic acid stains can be used in microscopy applications as well for determining live versus dead cells in addition to providing spatial context for other antigens of interest in microscopy.

Other probes useful in the assessment of viability status on live cells with the intent of fixing those cells prior to acquisition are the Zombie Dyes. This is a family of probes that are cell-impermeant unless the cell membrane is compromised as well. In this case, they will enter a dead cell and conjugate to intracellular proteins, creating a much more intense staining than that of the live cells.

- DRAQ5™ (Cat. No. 424101)
- DRAQ7™ (Cat. No. 424001)
- DAPI (Cat. No. 422801)
- Helix NP™ NIR (Cat. No. 425301)
- Helix NP™ Green (Cat. No. 425303)
- Helix NP™ Blue (Cat. No. 425305)
- CytoPhase™ Violet (Cat. No. 425701)
- Propidium Iodide (Cat. No. 421301)
- Zombie Dyes are available in UV, Violet, Aqua, Yellow, Green, Red and NIR channels.



Ramos cells treated with 5 µM CytoPhase™ Violet dye for 90 minutes at 37°C. Cells were then acquired on a flow cytometer equipped with a 405 nm laser with a 450/50 bandpass filter.



One day old splenocytes were stained with Zombie Aqua™ and analyzed without fixation (blue) or analyzed after fixation and permeabilization (red). Cells alone without Zombie Aqua™ staining are indicated in black.

Annexin V

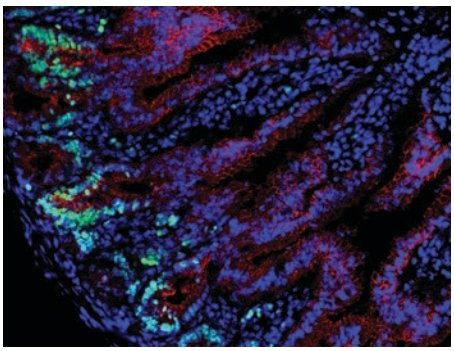
Apoptosis and programmed cell death are often the function of important oncogenes, regulating the arrest and removal of aberrantly proliferating cells. Annexin V is the most common probe used in detecting apoptosis. It is a protein that binds to phosphatidylserine (PS) in a calcium-dependent manner. PS residues will translocate to the outer leaflet of the plasma membrane in cells, undergoing membrane asymmetry associated with apoptosis. Annexin V alone will not indicate apoptosis since it will also detect intracellularly faced PS residues in dead cells once membrane integrity is lost. Therefore it must be used in conjunction with other probes that will assess live/dead status like the Zombie dyes or impermeant nucleic acid stains like the Helix NP™ family. Annexin V conjugates come in an array of spectral options, including BV421™, BV510™, PE, FITC and Alexa Fluor® 647.

Cell Vitality and Long-Term Cell Tracking

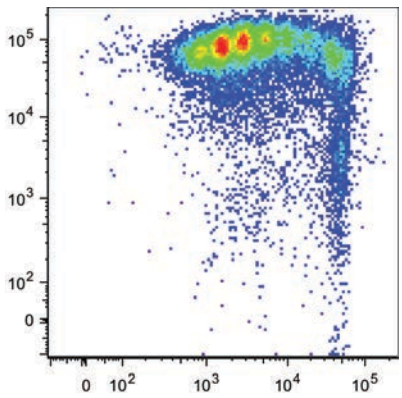
Esterase probes can be useful in immuno-oncology, both as another indicator of cell health and vitality, and for short and long-term cell tracking. Calcein-AM, Calcein Violet-AM, CFDA-SE and Tag-it Violet™ are fluorogenic esterase substrates that indicate not only that a cell is alive, but that it is also healthy, displaying an abundance of intracellular esterase activity. As cells enter and progress through apoptosis, esterase activity will diminish until only residual enzyme is left upon complete cellular death. CFDA-SE and Tag-it Violet™ can also be used to detect the vitality of esterase activity if the cell sample will need to be fixed prior to analysis or imaging. If the cell sample is already fixed, the Ki-67 antibody and BrdU are useful in determining which cells were proliferating at the time of fixation.

- Calcein-AM (Cat. No. 425201) and Calcein Violet-AM (Cat. No. 425203) can be used either as viability and vitality indicators or short-term cell trackers. However, the labeled cell will eventually pump out this probe, as its retention is only passive.
- CFDA-SE (CFSE: Cat No. 423801) is a classical, cell-permeant, long-term tracking dye that can be used for cell proliferation and tracking in microscopy and generational analysis in flow cytometry. CFDA-SE can also be used to identify vital, healthy live cells in culture prior to fixation and permeabilization.
- Tag-it Violet™ (Cat No. 425101) is an alternative to CFSE that excites at 405 nm and emits at 450 nm. It can be used in all the same applications of tracking, proliferation and generational analysis as CFSE and provides an additional color option that helps when tracking two populations of cells.
- Anti-Ki-67 antibodies are also available in several fluorescent conjugates, allowing you to identify proliferating cells post-fixation.
- BrdU is a bromine-modified nucleotide analog that when available in the presence of dividing cells, can become incorporated into newly replicated DNA. Detection requires fixation and permeabilization of the cells or tissue or use of an anti-BrdU antibody available in an array of fluorescent conjugates.

Find out more about our probes for proliferation and cell tracking at:
biolegend.com/cell_health_proliferation



C57BL/6 mouse frozen intestine section was fixed, permeabilized and blocked, then stained with Ki-67 (clone 11F6) Alexa Fluor® 488 (green) and E-cadherin (clone DECMA-1) Alexa Fluor® 594 (red). Nuclei were counterstained with DAPI (blue).



Human peripheral blood mononuclear cells were stained with Tag-it Violet™ Proliferation and Cell Tracking dye, and then stimulated with PHA for four days. On day four, cells were harvested, stained with CD3-PE and the Tag-it Violet™ signal was analyzed by flow cytometry

Contact BioLegend

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