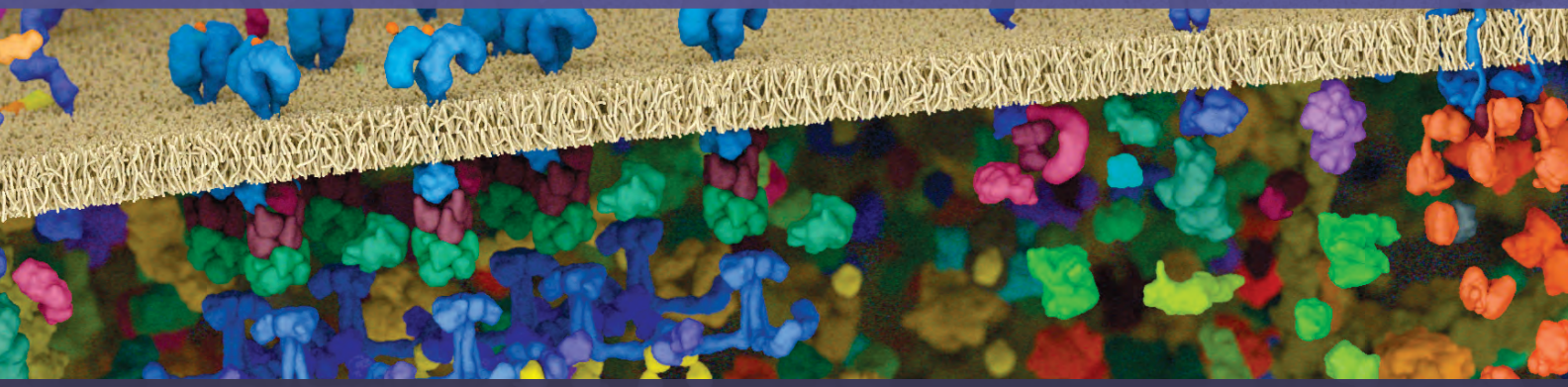

ANTIBODIES, KITS, AND REAGENTS FOR THE STUDY OF

CANCER IMMUNOLOGY

Cell Signaling TECHNOLOGY®

Research Tools for the Study of Cancer Immunology

CST has antibodies, kits, and reagents

for each stage of the experimental process.

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Over 1000 unconjugated and directly conjugated primary antibodies directed against more than 250 targets relevant to immunology and cancer immunology. The collection is continually expanding, so please check our website frequently for a complete, up-to-date list.
www.cellsignal.com/immuno_newproducts

Kits

Antibody Sampler Kits and Antibody Array Kits: provide a cost-effective way for analyzing multiple nodes in a pathway of interest or modification sites within a protein of interest.

Immunohistochemistry Kits: include IHC validated antibodies and signal enhancing companion products to bolster sensitivity of target detection.

ELISA Kits: allow for specific analyte detection that enables simple high-throughput analysis.

Chromatin IP Kits and Reagents: include SimpleChIP® and SimpleChIP® Plus kits, ChIP validated antibodies, control PCR primers, and companion products needed to perform successful ChIP assays.

PTMScan® Kits and Services: utilize motif antibodies and LC-MS/MS technology to generate quantitative profiles of hundreds to thousands of proteins containing a particular type of post-translational modification.

Experimental Controls

Isotype controls: control antibodies that are used to estimate non-specific binding of test primary antibodies due to Fc receptor binding and other protein-protein interactions.

SignalSlide® IHC controls: slides with formalin fixed paraffin embedded cell pellets that are verified to either express (positive control) or lack (negative control) the target of interest.

SignalSilence® siRNA: rigorously validated siRNAs that can be used to selectively reduce the expression of a target of interest.

Blocking Peptides: synthetic peptides that specifically bind the antibody against which they were designed and block further antibody binding, confirming antibody specificity and eliminating concerns about non-specific binding.

Companion Products

Secondary antibodies (conjugated to Alexa Fluor®, DyLight™ series, PE, Pacific Blue™, HRP and others), loading controls, buffers, dyes, detection reagents, protease and phosphatase inhibitors, and peptide substrates are available to support your workflow.

Chemical Modulators

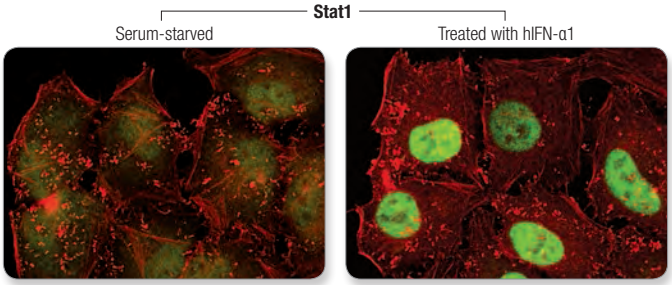
Growth factors and cytokines can be used for treatment of cells in order to stimulate or inhibit activation, phosphorylation, proliferation, and differentiation events.

Custom Products

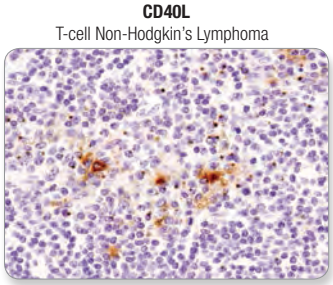
Our customs department will work with you if you require a product in a specific size, conjugation format, or formulation for your particular assay platform, or if you need a product validated for a specific measure or assay.

Workflow Solutions to Advance your Research

IF and IHC validated antibodies to assess the subcellular localization and spatial context of key immune cell targets

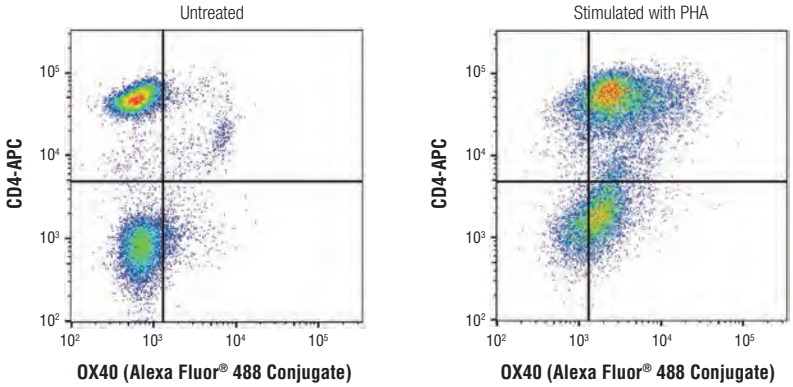


Stat1 (D1K9Y) Rabbit mAb #14994: Confocal IF analysis of HeLa cells serum-starved overnight (left) or treated with Human Interferon-α1 (hIFN-α1) #8927 (1,000 units/ml, 30 min; right), using #14994 (green) and β-Actin (8H10D10) Mouse mAb #3700 (red).



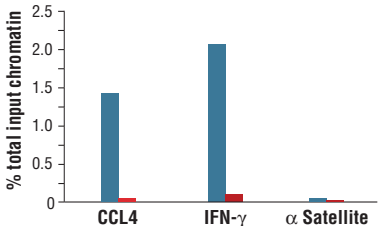
CD40 Ligand (D5J9Y) Rabbit mAb #15094: IHC analysis of paraffin-embedded human T-cell non-Hodgkin's lymphoma using #15094.

Flow Cytometry validated antibodies to allow for simultaneous detection of surface and intracellular targets



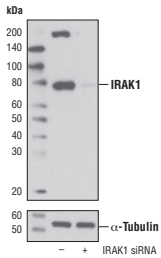
OX40 (D1S6L) Rabbit mAb (Alexa Fluor® 488 Conjugate) #15135: Flow cytometric analysis of human PBMCs, untreated (left) or PHA-treated (1 µg/ml, 48 hr, 37ºC; right), using #15135, which recognizes the intracellular domain of human OX40, and co-stained with an anti-human CD4 antibody.

ChIP validated antibodies and kits to examine protein-DNA interactions

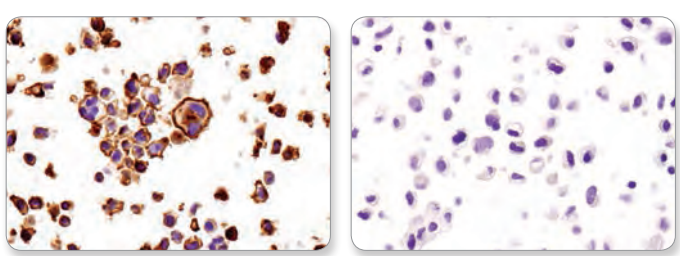


T-bet/TBX21 (D6N8B) XP® Rabbit mAb #13232: Chromatin IP was performed with cross-linked chromatin from 4 x 10⁶ NK-92 cells and either 10 µl of #13232 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using human CCL4 promoter primers, SimpleChIP® Human IFN-γ Promoter Primers #13051, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA is represented as a percentage of total input chromatin.

Experimental controls to help assess assay performance



SignalSilence® IRAK1 siRNA I #6253: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or #6253 (+) using IRAK1 (D51G7) XP® Rabbit mAb #4504 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The IRAK1 (D51G7) XP® Rabbit mAb confirms silencing of IRAK1 expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.

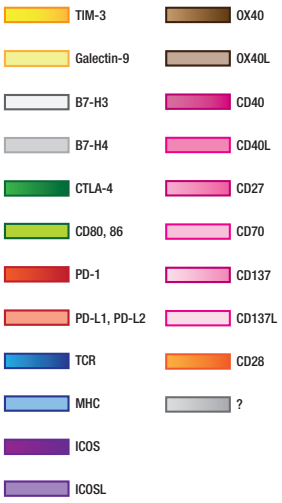


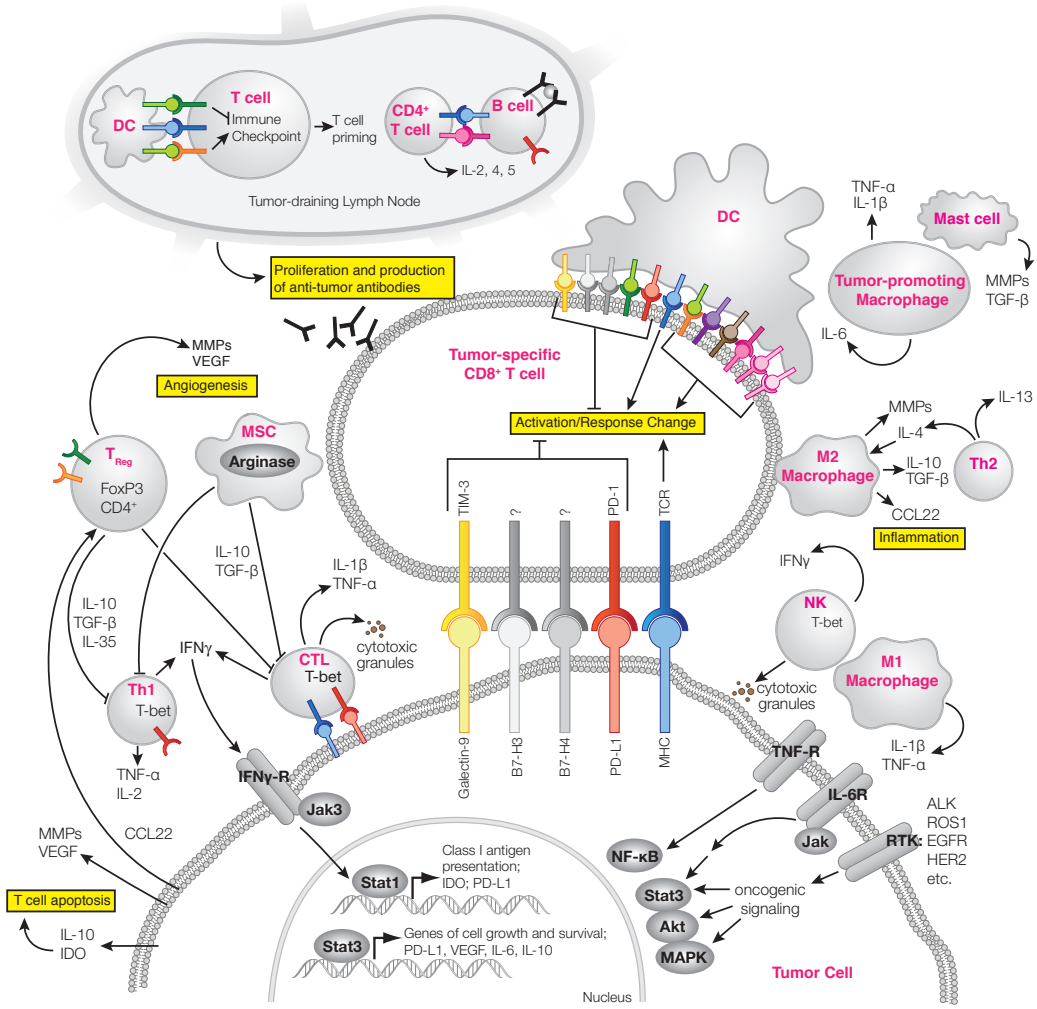
SignalSlide® PD-L1 IHC Controls #13747: IHC analysis of paraffin-embedded HDLM-2 (PD-L1 positive, left) and PC3 (PD-L1 negative, right) cell pellets using PD-L1 (E1L3N®) XP® Rabbit mAb #13684.

Immune Checkpoints and Cancer

	T-cell	APC
Co-stimulatory	CD28	B7-1 (CD80) or B7-2 (CD86)
	CD40L	CD40
	TLT-2?	B7-H3
	OX40 (CD134)	OX40L
	4-1BB (CD137)	4-1BBL
	ICOS	ICOSL
	GITR	GITRL
Co-inhibitory	CTLA-4	B7-1 (CD80) or B7-2 (CD86)
	PD-1	B7-H1 (PD-L1) or B7-DC (PD-L2)
	Unknown	B7-H3
	Unknown	B7-H4
	Unknown	VISTA
	VISTA	Unknown
	LAG-3	MHC-Class II
	TIM-3	Galectin-9

Table of stimulatory and inhibitory receptor-ligand complexes, which mediate activation or dampening of the T-cell response, respectively.





A successful immune response requires a refined balance of co-stimulatory and co-inhibitory inputs (see table, top left), which ensure detection and clearance of foreign material while staving off self-recognition. Fine-tuning this response, at least in part, involves regulation of T-cell function, which is governed by the activation of the T-cell receptor (TCR).

Immune checkpoint proteins belonging to the B7 family (B7-1, B7-2, B7-H1, B7-DC, B7-H3, B7-H4, HHLA, and others) are largely responsible for imposing negative inputs, which mitigate T-cell function post TCR activation. Tumor cells often evade immune detection by co-opting the inhibitory signaling axes, which normally hinder TCR activation. This is manifested as the upregulated expression of immune checkpoint ligands, such as PD-L1, PD-L2, and others, on the tumor cell surface. The binding of these ligands to corresponding receptors on T-cells acutely attenuates T-cell activation or response.

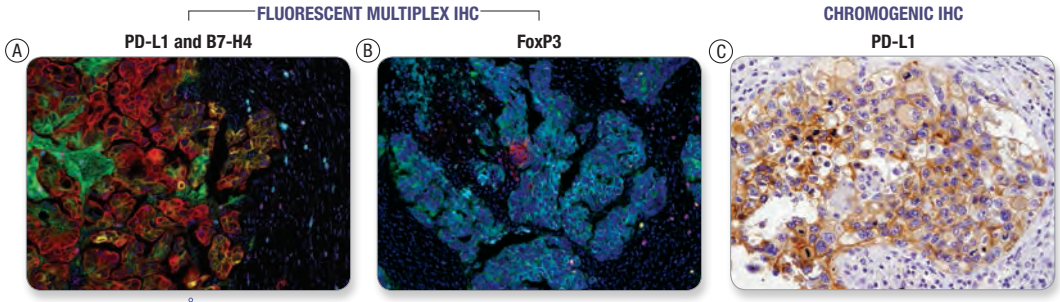
Select Reviews: Ceeraz, S. et al. (2013) *Trends Immunol.* 34, 556–563. Leung, J. and Suh, W.K. (2014) *Immune Network* 14, 265–276. Pardoll, D.M. (2012) *Nat. Rev. Cancer* 12, 252–264. Rozali, E.N. et al. (2012) *Clin. Dev. Immunol.* 2012, Article ID 656340.

Cancer Immunology Target List

For a complete product listing, including our expansive portfolio of modification-specific antibodies please visit our website.The targets in **bold** text are featured in this brochure.

Aiolos	CCL5	Fas	Granzyme A	Ikaros	**OX40**	SHPS1	Stat5	TNF-R1
B7-H3	**CD40 Ligand**	**FoxP3**	Granzyme B	IL-10	**PD-1**	SLP-76	Syndecan 1	TNF-R2
B7-H4	CIITA	Galectin-1	IDO	Lck	**PD-L1**	**Stat1**	**T-bet**	VISTA
Bcr	CTLA-4	Galectin-3	IFN-γ	NT5E/CD73	RANTES	Stat3	TNF-α	Zap-70

IHC validated antibodies to enable detection of immune checkpoint proteins in the context of three-dimensional tissue architecture



Fluorescent multiplex immunohistochemistry facilitates detection of the interface of tumor infiltrating lymphocytes (TILs) with cells of the tumor and its surrounding tissues aberrantly expressing immune checkpoint ligands such as B7-H4 and PD-L1.

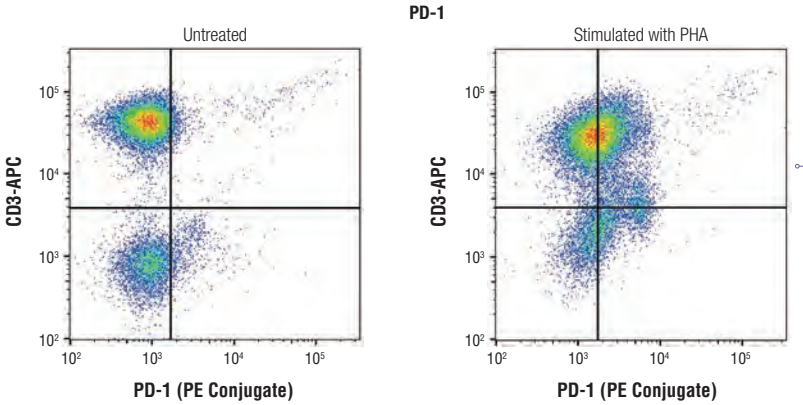
TILs have been observed in the context of several types of cancer and are often used as a correlative readout for clinical outcome (1,2). There is also evidence that PD-L1 expression within the tumor microenvironment is associated with increased clinical benefit (3).

1. Gooden, M.J. et al. (2011) *Br. J. Cancer* 105, 93–103.
2. Fridman, W.H. et al. (2012) *Nat. Rev. Cancer* 12, 298–306.
3. Mahoney, K.M. and Atkins, M.B. (2014) *Oncology* 28, 39–48.

PD-L1 (E1L3N®) XP® Rabbit mAb #13684
B7-H4 (D1M8I) XP® Rabbit mAb #14572
FoxP3 (D2W8E) Rabbit mAb (IHC Specific) #98377
Pan-Keratin (C11) Mouse mAb #4545

Fluorescent multiplex IHC analysis **(A, B)** of paraffin-embedded human ovarian tumor tissue. Figure **(A)** features #13684 (green), #14572 (red), #4545 (yellow), CD3 mAb (magenta), CD8 mAb (cyan). Figure **(B)** features #13684 (red), #14572 (green), #4545 (cyan), #98377 mAb (yellow), CD8 mAb (magenta). Blue pseudocolor = DAPI #4083 (fluorescent DNA dye). Detection of primary antibodies was achieved using a tyramide-based amplification approach. Chromogenic IHC analysis **(C)** of paraffin-embedded human lung carcinoma using #13684.

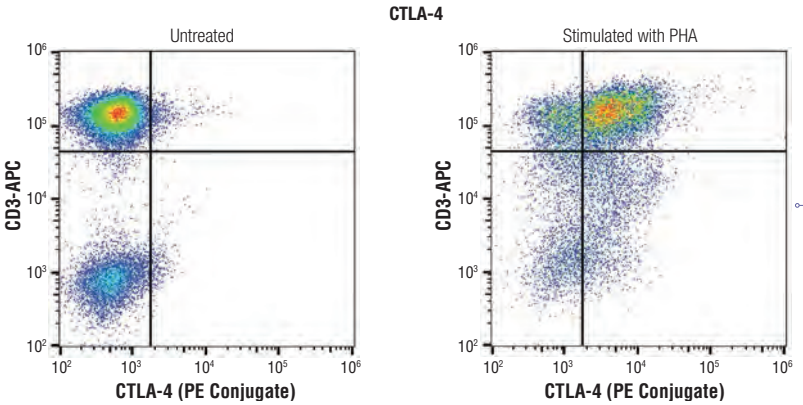
Flow Cytometry validated antibodies to detect co-expression of surface and intracellular targets and allow for phenotypic characterization



PD-1 (D3W4U) Rabbit mAb (PE Conjugate) #15151: Flow cytometric analysis of fixed and permeabilized human PBMCs using #15151, which recognizes the intracellular domain of PD-1, co-stained with CD3 APC. The cells were either untreated (left) or stimulated with PHA (1 µg/ml, 48 hr, 37°C; right).

Recent findings suggest that PD-1 expression levels within intratumoral T-cells, as measured by flow cytometry, can be correlated with prognostic outcome.

Yang, Z.Z et al. (2015) *Blood Cancer J.* 5. e281.



CTLA-4 (D4E9I) Rabbit mAb (PE Conjugate) #15132: Flow cytometric analysis of human PBMCs, untreated (left) or PHA-treated (1 µg/ml, 72 hr; right), using #15132 and co-stained with an anti-human CD3 antibody. Analysis was performed on cells in the lymphocyte gate.

Cytotoxic T-lymphocyte Antigen-4 (CTLA-4) was one of the first and most prominent immune checkpoint receptors to be targeted for immunotherapy.

The blockade of CTLA-4 with a neutralizing antibody (Ipilimumab) has shown immense promise as an anti-neoplastic strategy for melanoma and other cancers.

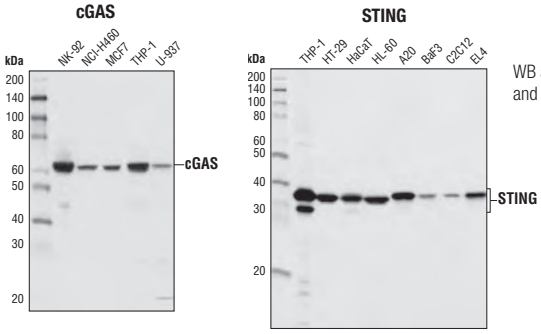
Buchbinder, E.I. and McDermott D.F. (2015) *Clin. Ther.* 37, 755–763.

Innate Immunity and Cancer

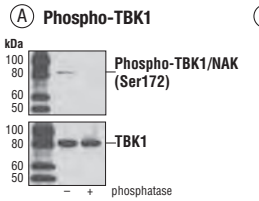
STING

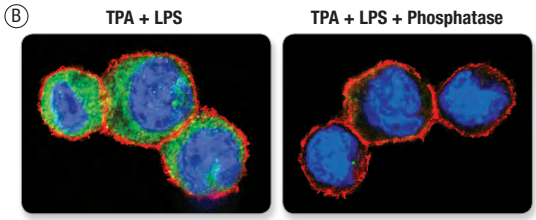
Stimulator of interferon genes (STING) is a key signaling molecule within the innate arm of the immune response, which enables detection of microbial as well as tumor-derived nucleic acids in the cytosol. Disruption of immune checkpoint pathways involving CTLA-4 and PD-1 is found to be therapeutically ineffective in mouse models lacking STING, indicating that activation of the STING pathway may be important for anti-neoplastic therapies involving checkpoint blockade.

References: Bronte, V. (2014) *Immunity* 51, 679–68. Burdette, D.L. and Vance, R.E. (2013), *Nat. Immunol.* 14, 19–26.

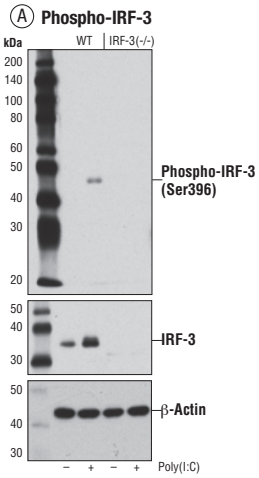


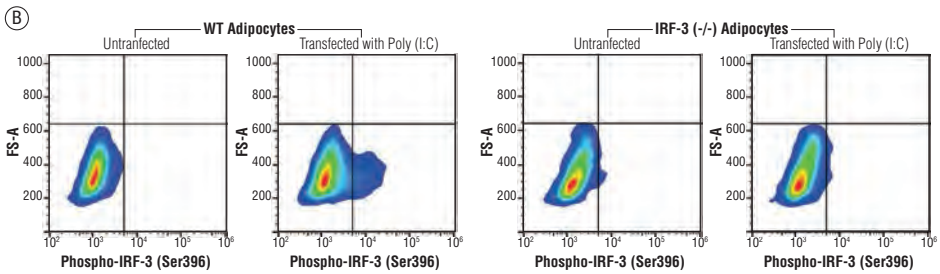
WB analysis of extracts from various cell lines using **cGAS (D1D3G) Rabbit mAb #15102** (left) and **STING (D2P2F) Rabbit mAb #13647** (right).



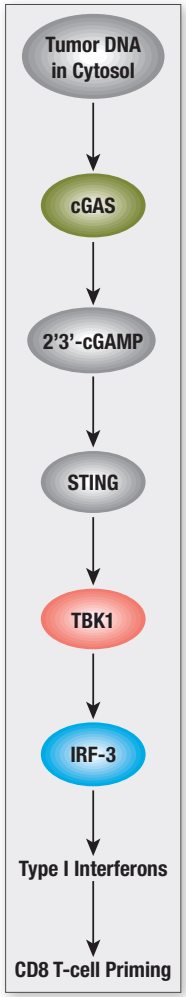


Phospho-TBK1/NAK (Ser172) (D52C2) XP® Rabbit mAb #5483: WB analysis **(A)** of extracts from THP-1 cells differentiated with TPA #4174 (80 nM, overnight) followed by treatment with LPS (1 µg/ml, 1 hr), with or without phosphatase treatment using #5483 (upper), or total **TBK1/NAK (D1B4) Rabbit mAb #3504** (lower). Confocal IF analysis **(B)** of THP-1 cells treated as described for figure **A** using #5483 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).





Phospho-IRF-3 (Ser396) (D601M) Rabbit mAb #29047: WB **(A)** and Flow cytometric **(B)** analyses of adipocytes from wild type (WT) and IRF-3 (-/-) mice, untransfected (-) or transfected with Poly (I:C) (2.5 µg/ml, 6 hr; +), using #29047. For WB analysis **IRF-3 (D83B9) Rabbit mAb #4302** (middle) and β-Actin (D6A8) Rabbit mAb #8457 (lower) were also used. For Flow cytometric analysis Anti-rabbit IgG (H+L), F(ab')$_2$ fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

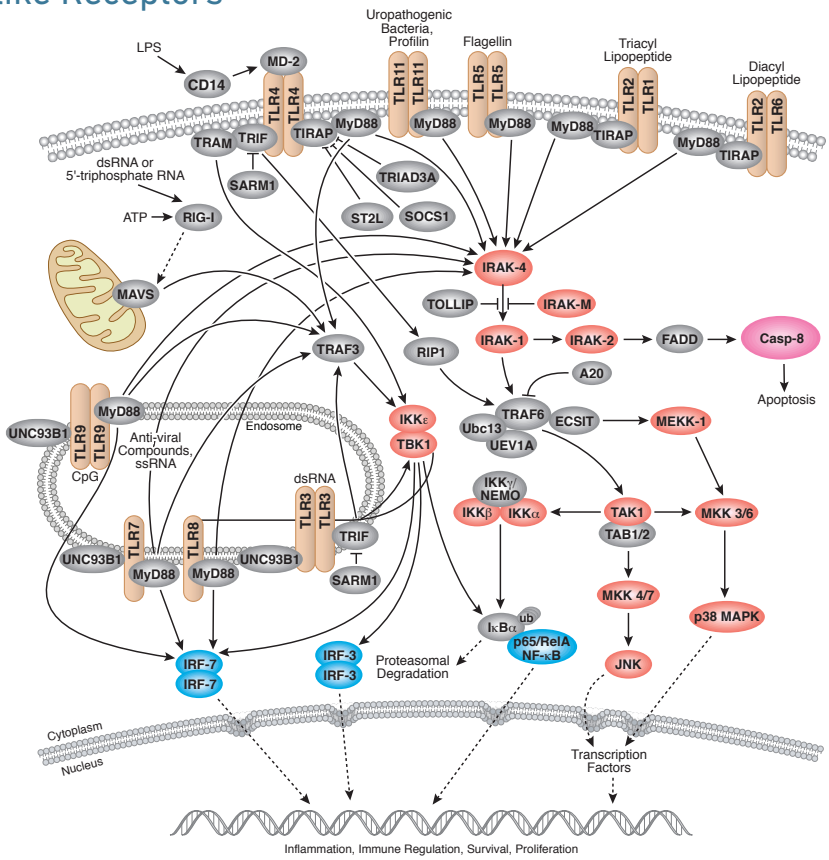


Innate Immunity Target List

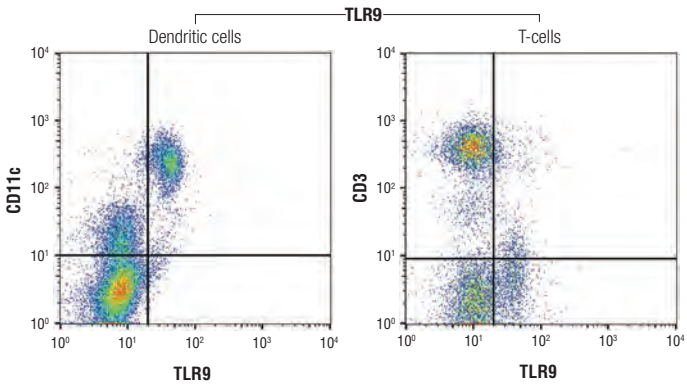
For a complete product listing, including our expansive portfolio of modification-specific antibodies please visit our website. The targets in **bold** text are featured in this brochure.

ADAR1	IFIT1	IRAK3	**IRF-3**	IRF-8	MAVS	NLRX1	Rig-I	Tollip	USP18	TLR2	TLR7
DC-SIGN	IRAK-M	IRAK4	IRF-4	**IRF-9**	MDA-5	Nod1	SAMHD1	TREX1	**TBK1**	TLR3	TLR8
Dectin-1	IRAK1	IRF-1	IRF-5	ISG15	MyD88	PACT	SINTBAD	TRIF	TIRAP	TLR4	**TLR9**
cGas	IRAK2	IRF-2	IRF-7	ITCH	NDP52	PKR	**STING**	TRIM25	TLR1	TRR6	

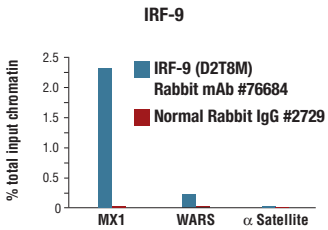
Toll-like Receptors



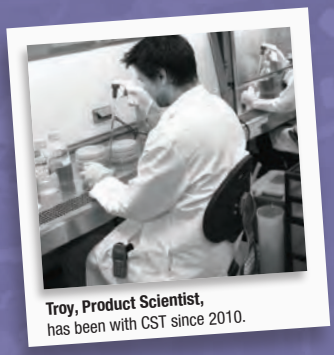
Toll-like receptors (TLRs) recognize distinct pathogen-associated molecular patterns (PAMPs) and play an integral role in the innate immune response. They participate in the first line of defense against invading pathogens and play a significant role in inflammation, immune cell regulation, survival, and proliferation. Recent studies indicate that TLR expression is not restricted to cells of immune origin and that tumor cells can also bear functional TLRs suggesting they play a role in tumor immunogenicity. The modulation of the inflammatory response by TLRs is a key factor in, tumor development and progression, triggering both tumor-promoting and anti-tumor responses.



Toll-like Receptor 9 (D9M9H) XP® Rabbit mAb #13674: Flow cytometric analysis of human PBMCs using #13674 co-stained with either CD11c (left) or CD3 (right).



IRF-9/ISGF3γ (D2T8M) Rabbit mAb #76684: Chromatin IP was performed with cross-linked chromatin from 4 x 10^6 U266 cells treated with Human Interferon-α1 (hIFN-α1) #8927 (10 nM, 30 min) and either 10 µl of #76684 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human MX1 Promoter Primers #57949, human WARS intron 1 primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA is represented as a percentage of total input chromatin.



Troy, Product Scientist, has been with CST since 2010.

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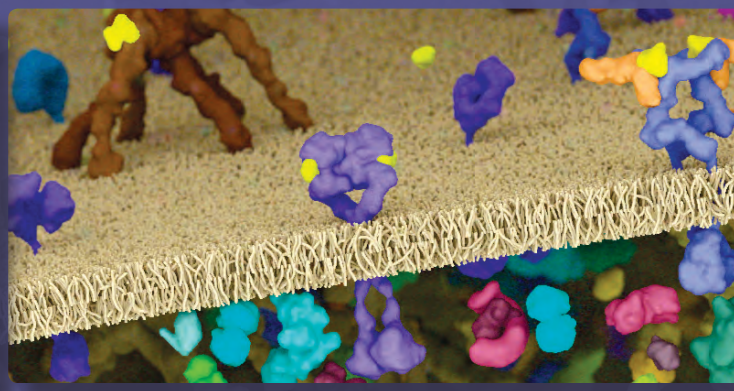

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COVER IMAGE: Molecular landscape depicting key receptor tyrosine kinase (RTK) signaling pathways. Structural data derived from the PDB and/or EMDB and modeled with Molecular Maya (mMaya).

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