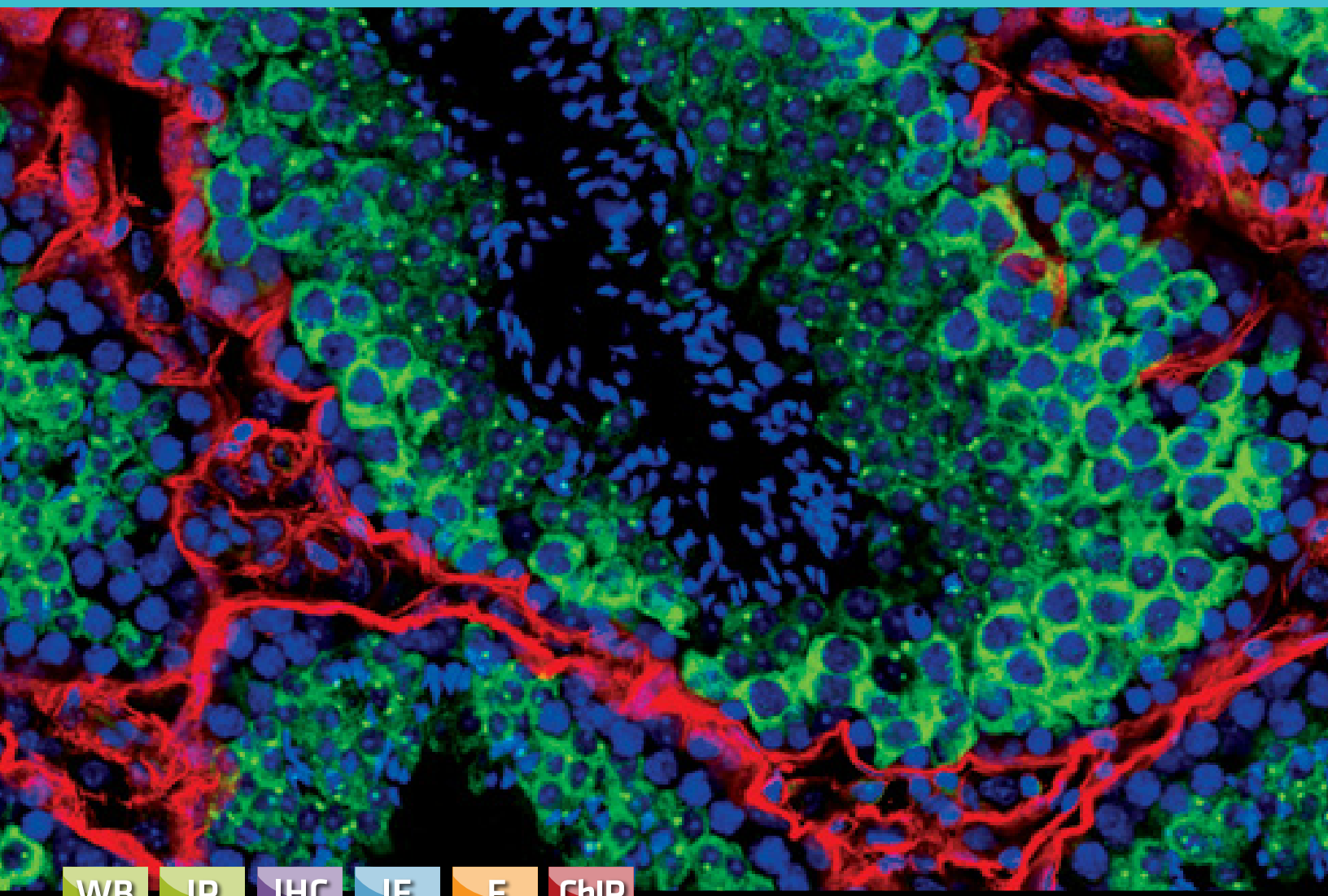
Antibody Specificity

Does your antibody measure up?



WB IP IHC IF F ChIP

The Story of a CST Antibody

At CST, we do things a bit differently . . .

Page 4

It's not you, it's your Antibody

The accuracy of your immunoassay results is dependent on the quality of the primary antibody used.

Page 9

Cell Signaling TECHNOLOGY®

PD-L1 (E1L3N™) XP® Rabbit mAb

from Cell Signaling Technology

WB IP IHC IF F

Reactivity: H

Target Specificity

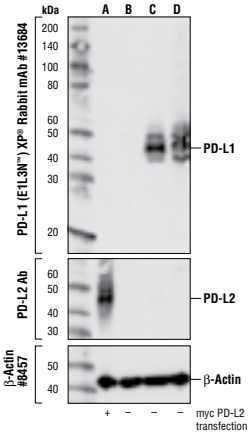
Recognizes PD-L1 and does not cross-react with other B7 family members

High Sensitivity

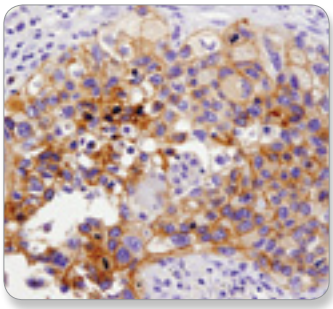
Detects endogenous levels of PD-L1 protein expression in human tissue

Validated in Multiple Research Applications

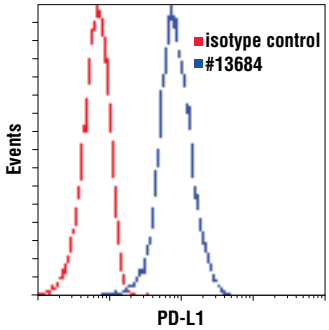
Demonstrates consistent, reliable results in multiple applications including flow cytometry



Western blot analysis of lysates from: **A**) COS cells (PD-L1 negative) transfected with PD-L2, **B**) COS cells mock transfected, **C**) KARPAS-299 cells, and **D**) SUP-M2 cells (PD-L1 positive) using the indicated antibodies.



Immunohistochemical (IHC) analysis of paraffin-embedded human lung carcinoma using #13684. IHC was performed using SignalStain® Antibody Diluent (#8112) and SignalStain® Boost IHC Detection Reagent (#8114).



Flow cytometric analysis of untreated SUP-M2 cells using #13684 (blue) compared to concentration matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody.

Background

Programmed cell death 1 ligand 1 (PD-L1, B7-H1, CD274) inhibits activated T cells by binding to its receptor, PD-1, on the surface of T cells. Research studies demonstrate that PD-L1 is present on the surface of tumor cells and suggest that the interaction between PD-L1 and PD-1 contributes to the malignancy of various tumors by helping them avoid immune detection (1-3). As a result, PD-L1 has garnered much attention both for its role in tumor immunology and as a potential therapeutic target (4).

References

1. Dong, H. et al. (2002) *Nat Med* 8, 793–800.
2. Thompson, R.H. et al. (2006) *Cancer Res.* 66, 3381–33815.
3. Pardoll, D.M. (2012) *Nat Rev Cancer* 12, 252–264.
4. Brahmer, J.R. et al. (2012) *N Engl J Med* 366, 2455–2465.

Visit our PD-L1 (E1L3N™) XP® Rabbit mAb #13684 product page for additional information. **www.cellsignal.com/learnPDL1**

Interested in Tumor Immunology? Order your complimentary poster at:

www.cellsignal.com/tumorimmuno

Antibody Specificity

Consequences for Research

Use of poorly validated antibodies results in wasted time and resources, including the expenditure of precious samples. Added to this, debate about the quality of research-use antibodies and the need for validation has intensified over the past few years (1-7). Numerous research manuscripts describing novel biomarkers have reportedly been withdrawn from publication due to mistaken conclusions based on assumed antibody specificity and inadequate controls (1). In one significant case, faulty antibody results from one research group lead to changes in the focus of type 1 diabetes research programs all over the world (7).

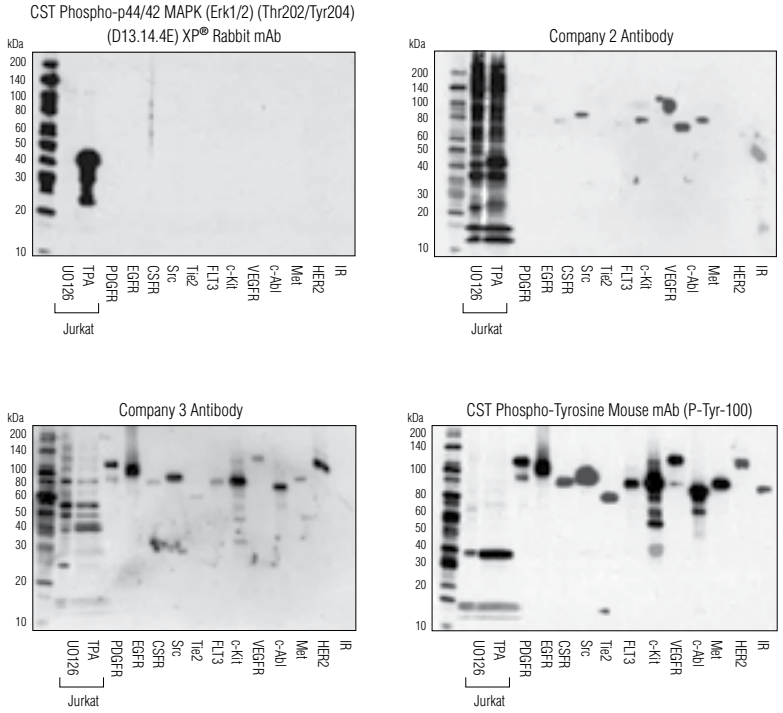
The Problem:

- Validating the specificity of your antibody is crucial to research success and reproducibility
- Documentation of specificity data is becoming a priority for publishers and reviewers
- Proper validation requires additional time and laboratory resources and may present a burden to some researchers

At CST, we believe that antibody validation is a shared responsibility between the researcher and the antibody supplier.

The CST Solution:

- **Stringently Validated Antibodies:** Each of our antibodies is rigorously validated in-house
- **From Scientist to Scientist:** CST antibodies are only released when our scientists are convinced of its specificity and sensitivity in the recommended applications
- **Expert Technical Support:** We offer you scientific technical support to make sure your antibody works the way it should



Western blot analysis using a panel of recombinant tyrosine-phosphorylated proteins shows no detectable cross-reactivity using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370, and significant cross-reactivity with other tyrosine phosphorylated proteins using both antibodies tested (Company 2 Antibody and Company 3 Antibody). Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 was used to demonstrate protein loading and verify molecular weight of the tagged recombinant proteins. These results demonstrate that #4370 displays exceptional specificity.

1. Saper CB (2005) **An open letter to our readers on the use of antibodies.** J. Comp. Neurol. 493(4), 477–8.
2. Saper CB (2009) **A guide to the perplexed on the specificity of antibodies.** J. Histochem. Cytochem. 57(1), 1–5.
3. Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D (2010) **Antibody validation.** BioTechniques 48(3), 197–209.
4. Welsh AW, Lannin DR, Young GS, Sherman ME, Figueroa JD, Henry NL, Ryden L, Kim C, Love RR, Schiff R, Rimm DL (2012) **Cytoplasmic estrogen receptor in breast cancer.** Clin. Cancer Res. 18(1), 118–26.
5. Lukinavičius G, Lavogina D, Gönczy P, Johnsson K (2013) **Commercial Cdk1 antibodies recognize the centrosomal protein Cep152.** BioTechniques 55(3), 111–4.
6. Blow, N (2013) Antibody Validation: Whose Job is it? **Biotechniques.com,** Oct 23.
7. Hansson SF, Korsgren S, Pontén F, Korsgren O (2013) **Enteroviruses and the pathogenesis of type 1 diabetes revisited: cross-reactivity of enterovirus capsid protein (VP1) antibodies with human mitochondrial proteins.** J. Pathol. 229(5), 719–28.

The Story of a CST Antibody

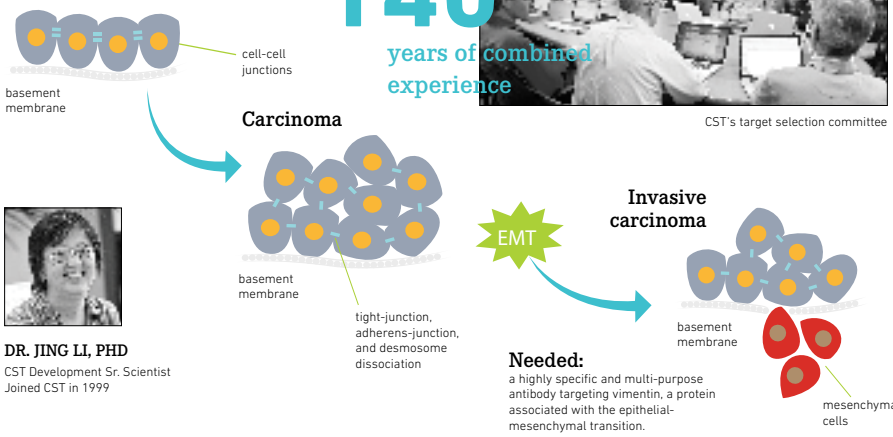
This is our story of one CST antibody's conception, development, production, and release for use in biomedical research.

How do we go from a **Scientific Question** and **Community Need** to delivering a high quality, validated, and technically supported **Antibody at Your Bench**?

See the full story at **www.cellsignal.com/soaa**

STEP 1 Target Selection:

Recognition of the scientific community's need for a cancer progression marker



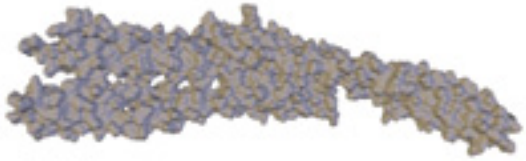
DR. JING LI, PHD
CST Development Sr. Scientist
Joined CST in 1999

Needed:
a highly specific and multi-purpose antibody targeting vimentin, a protein associated with the epithelial-mesenchymal transition.

140 years of combined experience

CST's target selection committee

STEP 2 Antigen Design & Clonal Expression

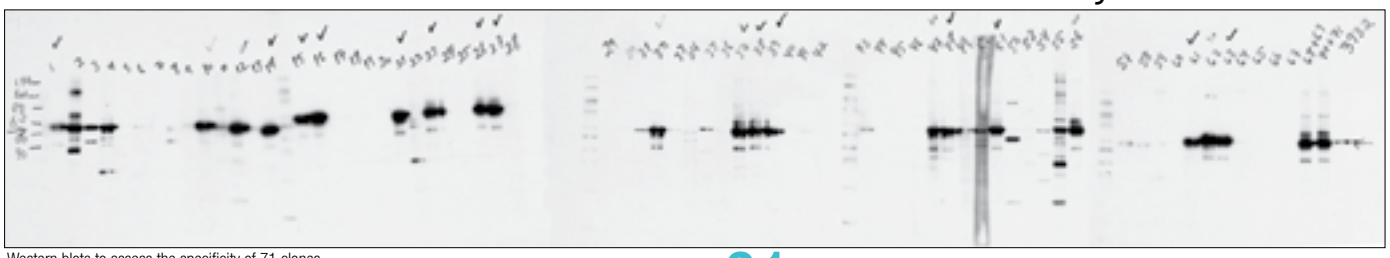


This 3-D molecular model of Vimentin can be viewed on Sketchfab.com

900+ clones screened for signal intensity by ELISA

STEP 3 Screening for Target Specificity

71 clones screened by western blot

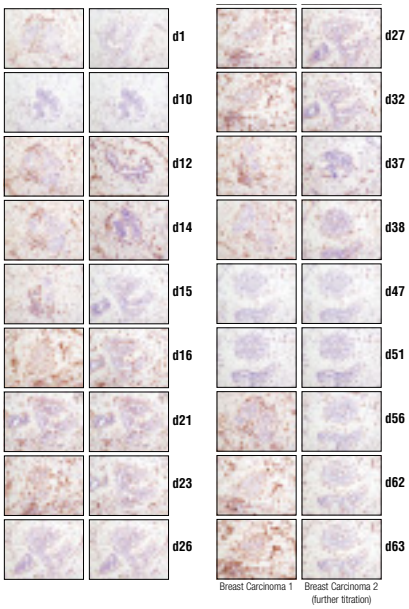


Western blots to assess the specificity of 71 clones.

21 clones isolated with appropriate specificity

STEP 4

IHC Screening for Localization



21 clones screened by IHC

4 clones isolated with robust, clean and specific staining

STEP 5

Final Lot Testing

1 highly-specific antibody passes CST's rigorous application validation tests: Vimentin (D21H3) XP® Rabbit mAb #5741

- ✓ WB Western Blotting
- IP Immunoprecipitation
- ✓ IHC Immunohistochemistry
- ✓ IF Immunofluorescence
- ✓ F Flow Cytometry
- ChIP Chromatin IP

STEP 6

Validation by Production

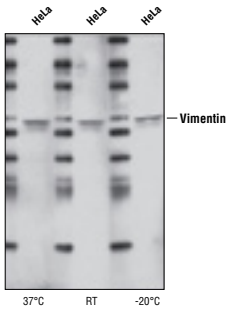
Technical Support from the same Scientists

On the phone, helping customers and at the bench, validating products...

STEP 7

Stability Testing

Room Temperature shipping



STEP 8

Antibody Release Form

Jing
CST Development Sr. Scientist,
Has been with CST since 1999.

Katie
CST IHC Group Leader
Has been with CST since 1999.

Gary
CST Development Sr. Group Leader,
Has been with CST since 2003.

Julie
CST Product Scientist,
Has been with CST since 2005.

Randy
CST Director of Cytometry,
Has been with CST since 2002.

45 peer-reviewed publications (and counting...)

The importance of a well-validated IHC antibody

Immunohistochemistry (IHC) is commonly used for morphological characterization of tumors or other tissue malignancies, and such samples are typically available in very limited amounts. Cell Signaling Technology® (CST™) antibodies for IHC Research analysis are thoroughly validated by the relevant methods, so you don't have to waste time or samples on validation. Our in-house IHC group strives to provide you with the most specific antibodies and lowest background possible.

Is your antibody specific?

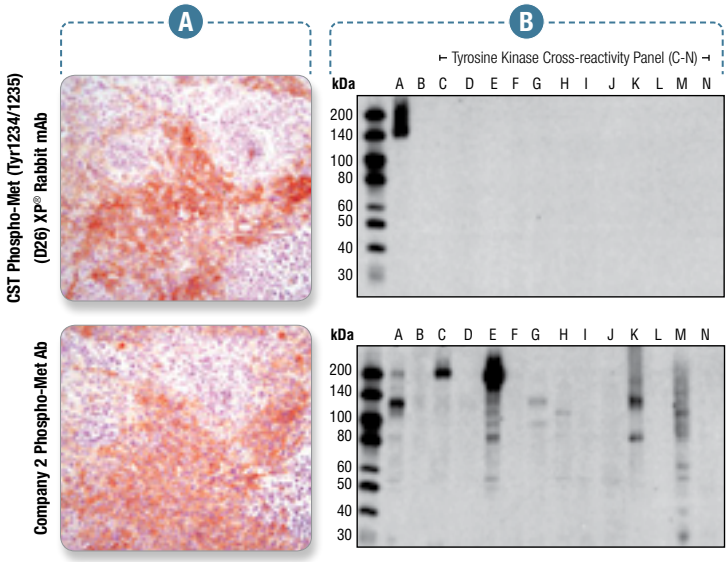
Have confidence in the specificity of your antibody, because CST has already evaluated each antibody by western blot and by IHC in paraffin-embedded control cell pellets as well as in biologically relevant samples.

Phospho-Met (Tyr1234/1235) (D26) XP® Rabbit mAb #3077: Comparison of CST #3077 (top) and another company's product (Company 2 Ab, bottom) on HCC827 xenograft **(A)** gives the appearance of specific staining for both products. Western blot analysis of various cell lines and treatments using #3077 and the Company 2 antibody show a single band at 145 kDa in HGF-stimulated, but not unstimulated A-431 cells **(B)**. #3077 demonstrated no cross-reactivity in control extracts treated with growth factors that activate other RTKs or that overexpress other RTKs or cytoplasmic tyrosine kinases (top). In contrast, the Company 2 phospho-Met antibody recognized several nonspecfic bands in these control experiments (bottom). Both membranes were developed on the same film with the same exposure time (10 sec).

Western blot analysis reveals that another company's phospho-Met antibody recognizes other phosphorylated RTKs, while #3077 specifically recognizes only phospho-Met.

Tyrosine Kinase Cross-reactivity Panel
Cell Lines & Treatments

A. A431 + HGF
B. A431 - HGF
C. NIH/3T3 + PDGF
D. NIH/3T3 - PDGF
E. A431 + EGF
F. COS/FGFR1
G. CHO/IRS1,IR + Insulin
H. K562
I. SUPM2
J. L540
K. NIH/3T3/src
L. GST-Tie-2
M. SEM
N. H526+SCF



Is your antibody supported by optimized reagents and protocols?

Ensure results are reproducible in your lab and others by using CST protocols and companion reagents that are optimized to work with your antibodies and targets.

When **PLK1 (208G4) Rabbit mAb #4513** was first released several years ago, our IHC group could not recommend its use for IHC based on its lack of staining. At that time, the standard reagents for IHC analysis were those listed below.

Diluent: TBST/5% NGS
Detection: biotin-based
Chromogen: NovaRed™

With the incorporation of **SignalStain® Antibody Diluent #8112**, we observed a slightly improved signal compared with our original conditions.

Diluent: #8112
Detection: biotin-based
Chromogen: NovaRed™

Changing the detection system to **SignalStain® Boost IHC Detection Reagent #8114** further improved the signal. However, based upon our rigorous standards, even a signal at this level did not warrant an IHC recommendation for this antibody.

Diluent: #8112
Detection: #8114
Chromogen: NovaRed™

Finally, when we changed the chromogen to **SignalStain® DAB Substrate Kit #8059**, we were able to produce a robust signal using this antibody, thus warranting an IHC recommendation. All changes in staining were achieved without altering the original antibody dilution.

Diluent: #8112
Detection: #8114
Chromogen: #8059

PLK1 (208G4) Rabbit mAb #4513: IHC analysis of paraffin-embedded human colon carcinoma using #4513 and various IHC reagents, as indicated.

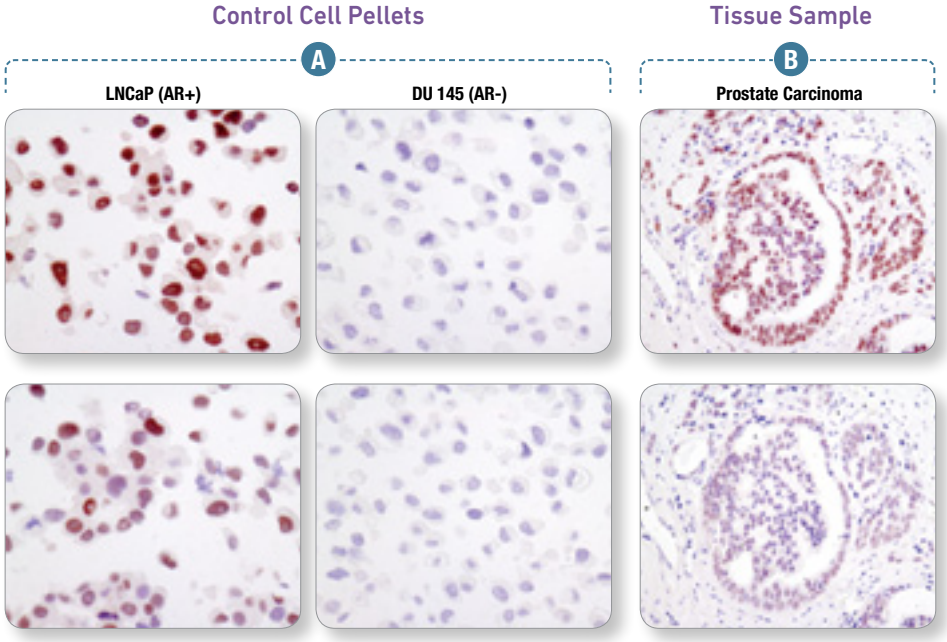
All improvements in staining were achieved solely by replacing reagents, without altering the original antibody dilution, demonstrating the impact of diluent, detection and chromogen regeants upon your IHC results.

Is your antibody as sensitive as you need?

Have confidence that your antibody is sensitive enough to specifically detect your target in your tissue samples, because CST has already evaluated staining on control cell pellets and biologically relevant samples.

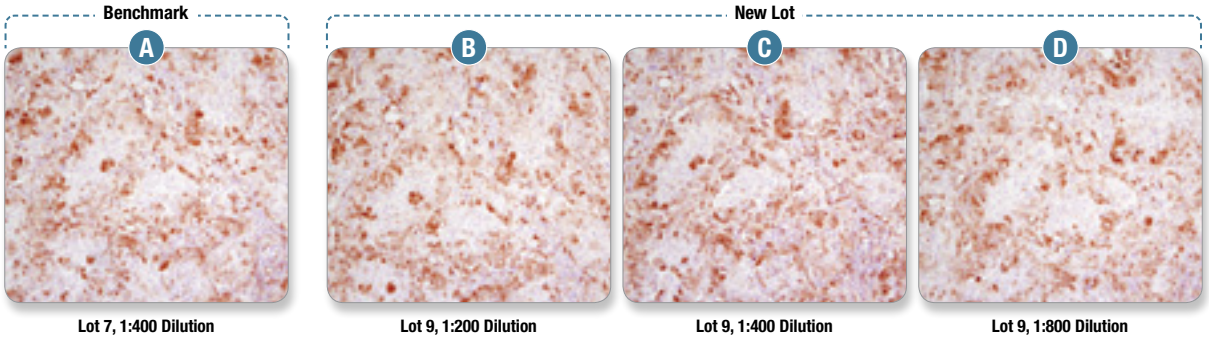
Androgen Receptor (D6F11) XP® Rabbit mAb #5153: IHC analysis of #5153 was compared to another company's IHC-approved androgen receptor mouse monoclonal antibody. The optimal dilution of each antibody was individually evaluated to optimize androgen receptor (AR) staining in AR expressing LNCaP cells and minimize nonspecific staining in AR null DU 145 cells **(A)**. The determined optimal dilution for each antibody was utilized in IHC analysis of paraffin-embedded human prostate carcinoma **(B)**.

At the determined optimal dilution for staining in AR expressing LNCaP cells and lack of nonspecific staining in AR null DU 145 cells, the other company's antibody fails to significantly stain tissue. The CST antibody accurately stains both cells and tissue.



Is your antibody performing consistently throughout your research?

Ensure reagents are reliable for the life of your project, because CST calibrates every new antibody lot with previous lots to minimize any variation.



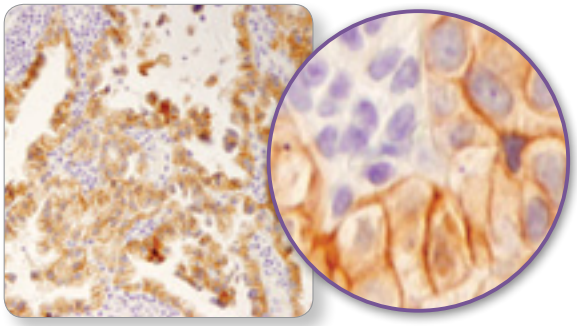
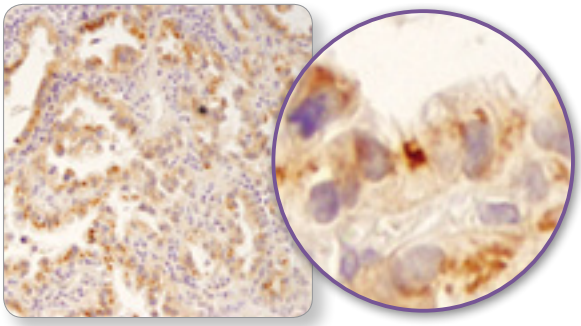
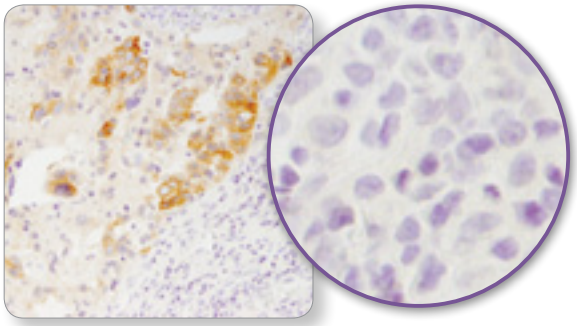
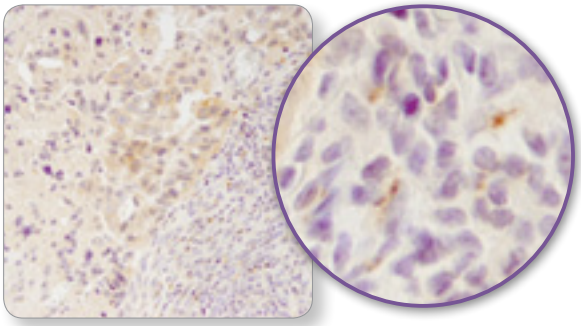
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370: IHC analysis of adjacent sections of paraffin-embedded human colon carcinoma using #4370 lot 7 at a 1:400 dilution **(A)**, #4370 lot 9 at a 1:200 dilution **(B)**, #4370 lot 9 at a 1:400 dilution **(C)** and #4370 lot 9 at a 1:800 dilution **(D)**. The recommended dilution for lot 9 remained 1:400.

Does your IHC antibody measure up?

Rigorously Validated IHC Antibodies Against High Impact Research Targets

from Cell Signaling Technology

Paraffin-embedded human lung and ovarian carcinoma tissues using CST™ HER3/ErbB3 (D22C5) XP® Rabbit mAb #12708 or another company's mouse monoclonal antibody.

	CST HER3/ErbB3 (D22C5) XP® Rabbit mAb	Company 2 Mouse Monoclonal Antibody
Lung Carcinoma	 Strong, specific plasma membrane-associated HER3 staining in the epithelium	 Non-specific, non-membranous staining in the epithelium
Ovarian Carcinoma	 Strong, specific signal in tumor cells, and no staining seen in stromal cells	 Weak signal in tumor cells, and non-specific staining in stromal cells

WB IP IHC IF F ChIP

It's not you, it's your Antibody

The accuracy of your immunoassay results is dependent on the quality of the primary antibody used.

CST Validation experiments

to ensure antibody target specificity:

1. **Detection of Endogenous Protein Levels**
   Testing samples from cell lines and/or tissues with known expression or absence of the protein of interest.

2. **Phospho-specificity**
   Antibody testing on lysates that are untreated compared to treatment with phosphatases.

3. **Biologically Relevant Treatments**
   Testing samples from cell lines that are treated with growth factors, cytokines, or chemical activators/inhibitors to knowingly modify target expression and/or modification.

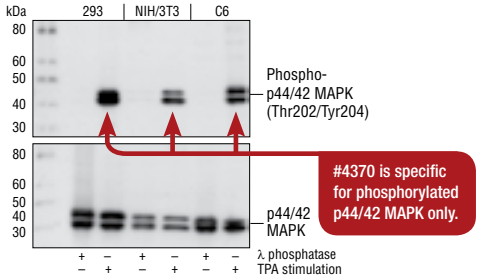
4. **siRNA Knockdown**
   Testing samples from cell lines transiently transfected with siRNA to knock down target protein expression

5. **Lot-to-Lot Consistency**
   All new antibody lots are compared to previous lots of the same antibody in parallel experiments

6. **Immunoprecipitation validation**
   Mouse or rabbit immunoglobulin (IgG) conjugated to beads in the absence of primary antibody ensure that non-specific binding to beads or the IgG is detected.

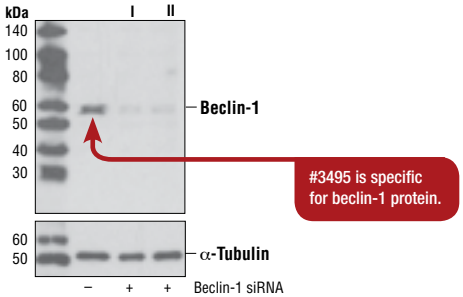
A control sample of 10% lysate demonstrates lysate integrity and provides a point of reference to evaluate the ability of the antibody to enrich the target protein.

Western Blot of phospho-specificity



Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370: WB analysis of extracts from 293, NIH/3T3, and C6 cells, treated with λ phosphatase (to inhibit phosphorylation) or TPA #4174 (to stimulate phosphorylation), using #4370 (upper), or p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).

siRNA knockdown to test specificity



Beclin-1 (D40C5) XP® Rabbit mAb #3495: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® Beclin-1 siRNA I #6222 (+) or SignalSilence® Beclin-1 siRNA II #6246 (+), using #3495 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The Beclin-1 (D40C5) XP® Rabbit mAb confirms silencing of Beclin-1 expression, while the α-Tubulin (11H10) Rabbit mAb is used to control for protein loading.

Intracellular Flow Cytometry

Validated antibodies

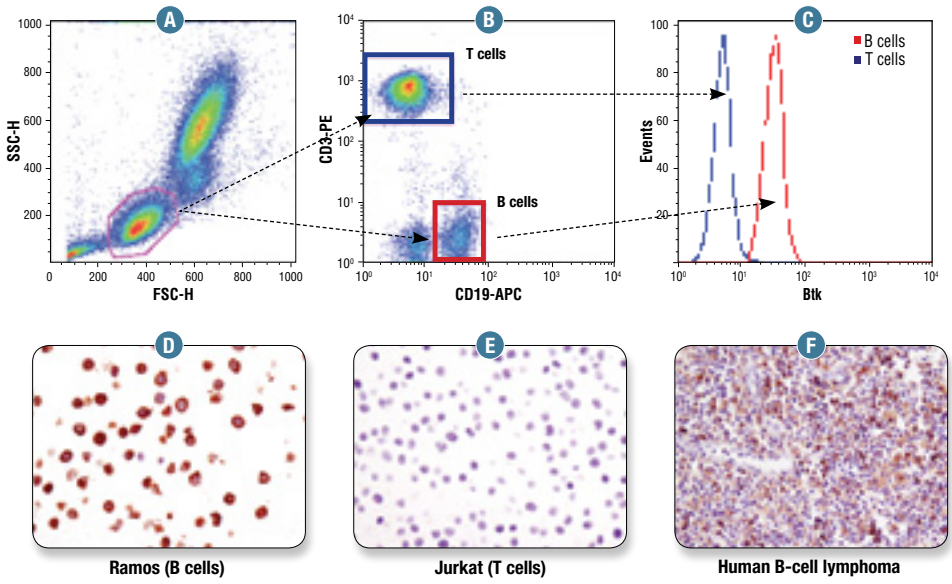
Cell Signaling Technology's dedicated Flow Cytometry group performs rigorous testing in biologically relevant models, ensuring specificity and optimal signal to noise for both purified and conjugated antibodies. Cross-platform validation further confirms antibody specificity, providing the highest quality reagents for flow cytometric analysis of mechanisms underlying cellular signaling.

Is your antibody specific?

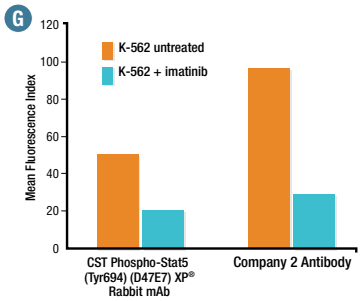
Have more confidence in the specificity of your antibody, because CST has already evaluated each antibody in biologically relevant samples by multiple methods.

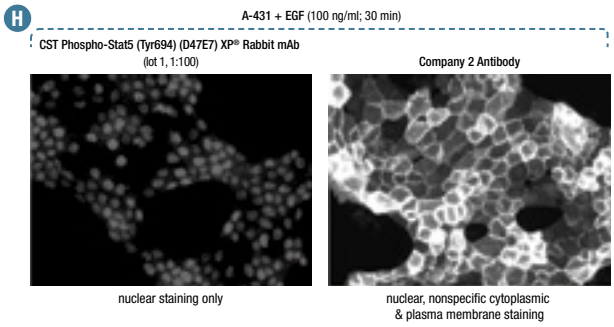
Btk is selectively expressed in B cells.

Btk (D3H5) Rabbit mAb #8547: Human whole blood was fixed and permeabilized as outlined in the Cell Signaling Technology Flow Alternate Protocol and stained using #8547. FSC & SSC were used to gate on live cells **(A)**. Samples were co-stained using CD3-PE and CD19-APC to distinguish live T and B cell subpopulations, respectively **(B)**. B (red) and T (blue) cell population gates were applied to a histogram depicting the mean fluorescence intensity of Btk **(C)**. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. IHC analysis of paraffin-embedded cell pellets and tissue **(D–F)**, using #8547.



Phospho-Stat5 (Tyr694) is localized to the nucleus.



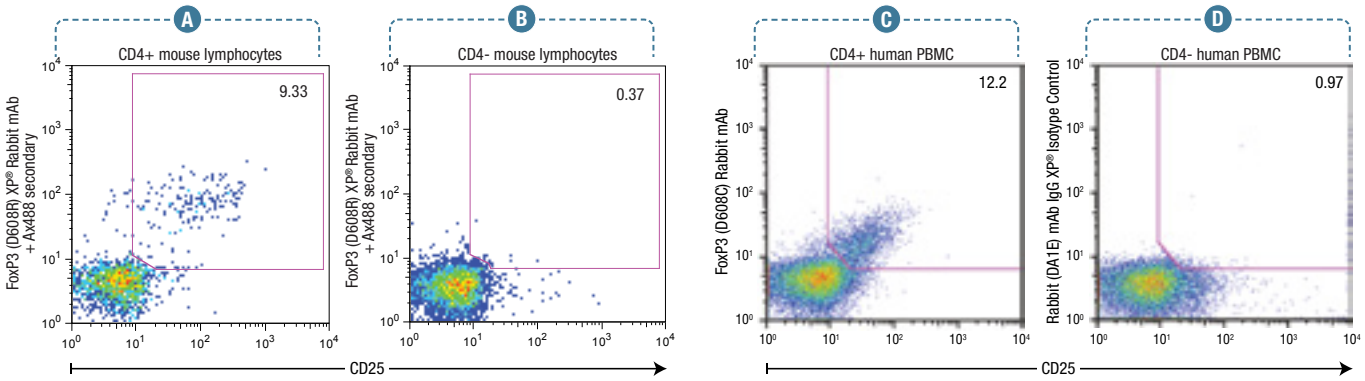


Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb #4322: Flow cytometric analysis to show a lower fold induction of Stat5 phosphorylation in response to imatinib treatment compared to another company's phospho-Stat5 (Tyr694) antibody **(G)**, but fluorescent immunocytochemical staining reveals that the other company's antibody inappropriately stains the cytoplasm and plasma membrane, while #4322 demonstrates only the appropriate nuclear staining **(H)**.

Is your antibody supported by optimized protocols?

Ensure accurate results in your lab by using protocols optimized to work with CST™ antibodies and your targets.

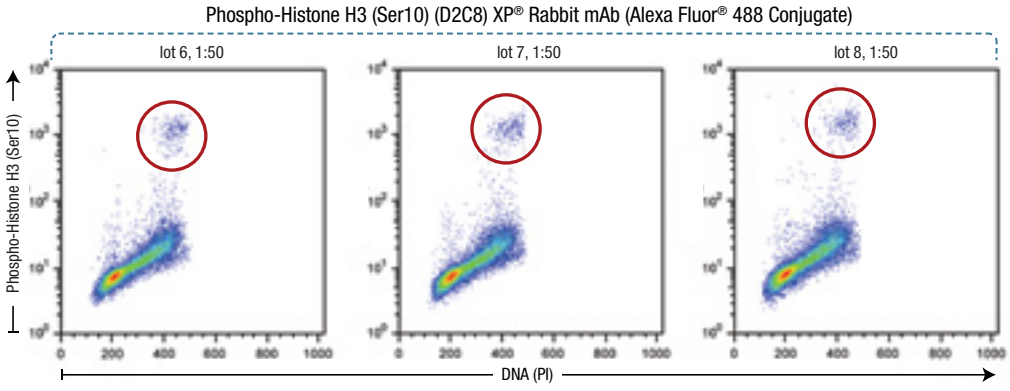
FoxP3 (D6O8R) XP® Rabbit mAb #12653 and FoxP3 (D608C) Rabbit mAb #12632 are each provided with individually optimized protocols, one for analyzing mouse samples and one for analyzing human samples.
The fixation and permeabilization steps of both protocols can be performed using standard lab reagents, so you won't need proprietary buffers and kits to conduct your experiments.



FoxP3 (D608R) XP® Rabbit mAb #12653: Flow cytometry analysis of murine spleen lymphocytes using a CD25 antibody and #12653. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. Analysis was performed on gated CD4+ lymphocytes **(A)** and CD4- lymphocytes **(B)** as an internal negative control. #12653 enables optimal detection of signal intensity and population frequency of FoxP3+/CD25+ T_{reg} cells. | **FoxP3 (D608C) Rabbit mAb #12632:** Flow cytometry analysis of human peripheral blood mononuclear cells gated on CD4+ lymphocytes, showing FoxP3 expression in CD25+ cells using #12632 **(C)**, and corresponding absence of signal in CD25+ cells using concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 **(D)**. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. FoxP3 (D6O8C) Rabbit mAb #12632 clearly defined the FoxP3+/CD25+ subset with regards to signal intensity and population frequency.

Is your antibody performing consistently throughout your research?

Ensure reagents are reliable for the life of your project, because CST calibrates every new antibody lot with previous lots to minimize any variation.



Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #3465: Flow cytometry analysis of Jurkat cells using multiple lots of #3465 compared to propidium iodide (DNA content). The recommended dilution of #3465 remained 1:50 for all lots compared.

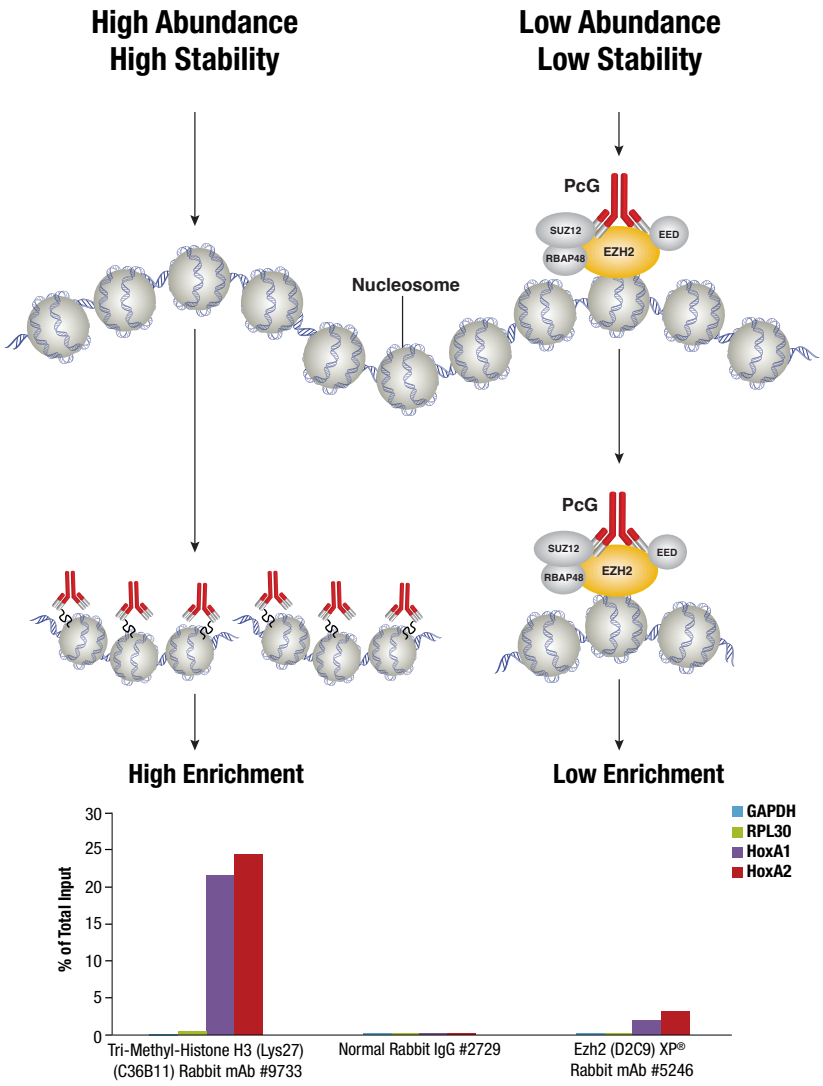
Optimized Reagents Matter

SimpleChIP® Plus Chromatin IP Kits and ChIP-validated Antibodies

The success or failure of a ChIP experiment is highly dependent on the integrity of the chromatin, the quality of the epitope, and the specificity of the antibody. Just as important is the inclusion of a control antibody that binds at the locus of interest and allows the investigator to confidently assess results. These components must be optimized to work together, especially when the target interaction is a low abundance, low stability event.

High abundance, very stable protein-DNA interactions like those between histones and DNA, occur frequently enough that they may still be detected even if the integrity of the DNA or protein epitopes has been compromised, or if the signal to noise ratio of the antibody is low.

Low abundance, less stable interactions such as the binding of polycomb group proteins (e.g., Ezh2) to specific genes, may fall under the detection threshold if the protocol fails to safeguard the integrity of the protein and the DNA, or if it relies on an antibody that is not highly specific to the target of interest.



For more information on CST's ChIP Kits and protocols, including data on enzymatic digestion versus sonication please contact us to request a SimpleChip Brochure.

Antibody Specificity: CST™ ChIP-Validated Antibodies

Antibodies that non-specifically bind unintended targets increase the background noise, making it more difficult to detect low abundance interactions.

CST offers antibodies that have been validated to work in ChIP applications, using the same rigorous standards we apply to all our antibodies.

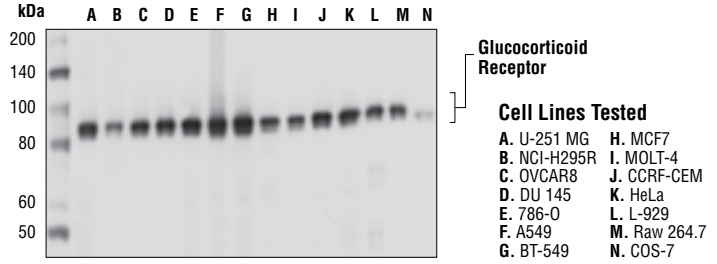
Please visit **www.cellsignal.com/cstchipab** for a full list of ChIP validated antibodies.

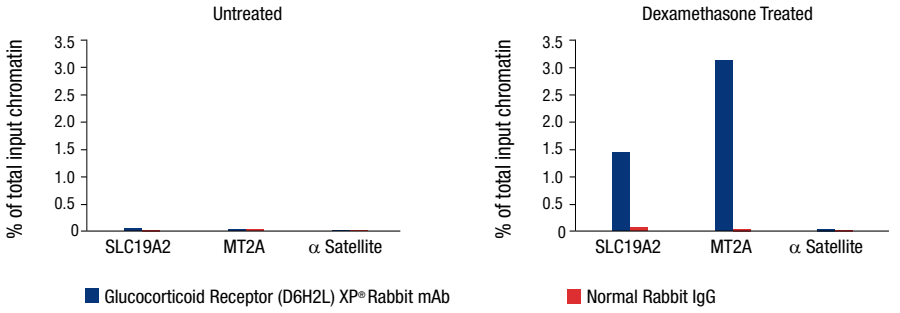
Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041 showed efficient target enrichment only when the cells were treated with dexamethasone, indicating the antibody is highly specific for the target of interest.

Western blot analysis of extracts from various cell lines using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041(upper).

A549 cells were cultured in media with 5% charcoal-stripped FBS for 3 d and then either untreated (left panel) or dexamethasone-treated (100 nM, 1 hr; right panel). Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 cells and 10 µl of Glucocorticoid Receptor (D6H2L) Rabbit mAb #12041or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human SLC19A2 Promoter Primers #7681, human MT2A promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as percent of the total input chromatin. (lower)

Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041





SimpleChIP® Plus Chromatin IP Kits from CST detect endogenous protein-DNA interactions in cultured cells and tissue samples.

These kits contain all reagents necessary to perform enzymatic digestion-based chromatin immunoprecipitation (ChIP) experiments quickly and easily, as well as positive and negative controls that allow you to be confident in your results. These kits are available with either Protein G agarose or Protein G magnetic beads and contain all buffers and reagents needed to perform up to 30 ChIP assays.

Each kit is designed to optimize:

❶ Chromatin Integrity

Enzymatic digestion gently fragments the chromatin, protecting the integrity of the protein and the DNA.

❷ Assay Reliability

The Histone H3 antibody is a universal control for tracking assay efficiency and reagent performance.

❸ Antibody Specificity

CST ChIP-validated antibodies are rigorously tested and validated, ensuring they will specifically bind to their intended target.

SimpleChIP® Plus Enzymatic Chromatin IP Kits

Cat.#	Name	Application	Reactivity
#9004	SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads)	ChIP	H, M, R, Mk
#9005	SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads)	ChIP	H, M, R, Mk

PTMScan® Discovery Kits and Services

Post Translational Modification Proteomics

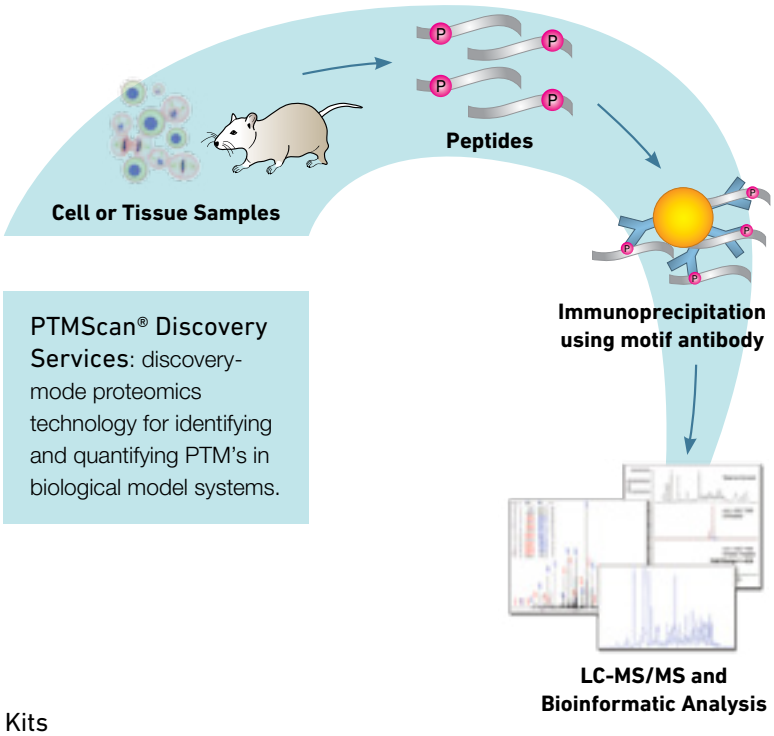
PTMScan® Technology, proprietary to Cell Signaling Technology® (CST™), utilizes the specificity of PTM-specific and motif antibodies to enrich target peptides from the background of non-modified endogenous peptides enabling the identification of modifed proteins that otherwise may not be detected through tandem mass spectrometry analysis (LC-MS/MS).

How can PTMScan help you?

- Allows for the identification and quantification of novel or low abundance post-translational modifications including phosphorylation, ubiquitination, acetylation, methylation, and succinylation by using PTM-specific and motif antibodies for immunoaffinity enrichment.
- Enables identification of novel PTM events in research involving disease state or drug treatment response
- PTMScan proteomics technology can be applied to many biological systems and species to support diverse research interests
- Expert technical service is provided by CST proteomics scientists throughout your experiment

PTMScan® Discovery Services

- AcetylScan® Proteomics Service
- UbiScan® Proteomics Service
- Ser/Thr PhosphoScan® Proteomics Service
- Tyrosine PhosphoScan® Proteomics Service
- Mono-MethylScan™ Proteomics Service
- Di-MethylScan™ Proteomics Service
- SuccinylScan™ Proteomics Service



PTMScan® Discovery Services: discovery-mode proteomics technology for identifying and quantifying PTM's in biological model systems.

Available Kits

Cat.#	PTMScan® Kit Description	Recommended Protease Treatment
#5563	PTMScan® Phospho-Akt Substrate Motif mAb 2 (RXRXXS*/T*) Kit	LysC*
#5561	PTMScan® Phospho-Akt Substrate Motif mAb 1 (RXXS*/T*) Kit	LysC*
#5564	PTMScan® Phospho-AMPK Substrate Motif (LXRXXS*/T*) Kit	LysC*
#12267	PTMScan® Phospho-ATM/ATR Substrate Motif [pSQ] Kit	Trypsin
#12235	PTMScan® Mono-Methyl Arginine Motif [mme-RG] Kit	Trypsin*
#13474	PTMScan® Asymmetric Di-Methyl Arginine Motif [adme-R] Kit	Trypsin*
#13563	PTMScan® Symmetric Di-Methyl Arginine Motif [sdme-RG] Kit	Trypsin*
#12810	PTMScan® Cleaved Caspase Substrate Motif [DE(T/S/A)D] Kit	Trypsin
#12170	PTMScan® Phospho-CK2 Substrate Motif (S*/T*DXE) Kit	Trypsin
#13416	PTMScan® Acetyl-Lysine Motif [Ac-K] Kit	Trypsin
#4652	PTMScan® Phospho-MAPK/CDK Substrate Motif (PXS*P and S*PXK/R) Kit	Trypsin
#5565	PTMScan® Phospho-PKA Substrate Motif (RRXS*/T*) Kit	LysC*
#5636	PTMScan® Phospho-Tyrosine Mouse mAb (P-Tyr-100) Kit	Trypsin
#8803	PTMScan® Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit	Trypsin
#5567	PTMScan® Phospho-T*PP Motif (T*PP) XP® Kit	Trypsin
#5566	PTMScan® Phospho-ST*P Motif (ST*P) XP® Kit	Trypsin
#13764	PTMScan® Succinyl-Lysine Motif [Succ-K] Kit	Trypsin
#5562	PTMScan® Ubiquitin Remnant Motif (K-ε-GG) Kit	Trypsin

*For LysC-digested material, there is a good second digestion performed after the StageTip purification of enriched peptides (see the protocol after StageTip Purification).

Visit **www.cellsignal.com/cstptm** for additional information.

PathScan® Antibody Array Kits

Multiplex Format

Analyze multiple targets simultaneously

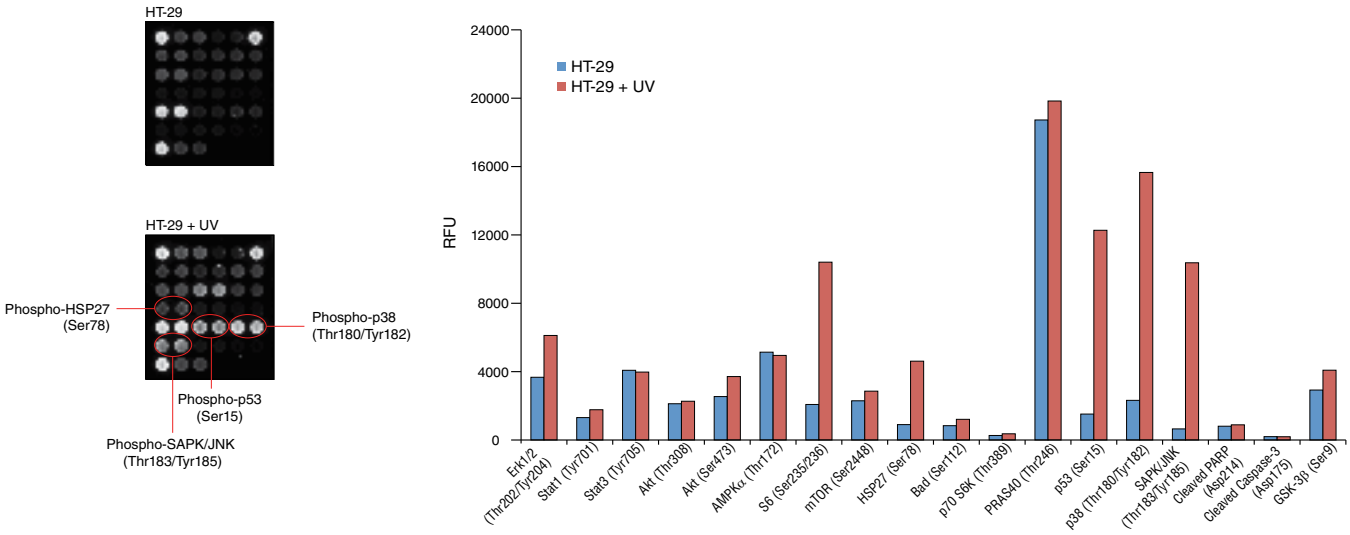
Multiple Array Pads

Test up to 32 experimental variables in parallel generating up to 608 datapoints per kit for rich experimental design

Low Sample Volume

Use as little as 50µl of sample (0.2-0.5 mg/ml total protein) per pad

Example Experiment: PathScan® Intracellular Signaling Array Kit (Fluorescent Readout)



HT-29 cells were grown to 80% confluency and then either untreated or UV-irradiated and allowed to recover for 60 min. Cell extracts were prepared and analyzed using the PathScan® Intracellular Signaling Array Kit (Fluorescent Readout) #7744. Panel A shows images that were acquired using the LI-COR® Biosciences Odyssey® imaging system. Panel B shows quantification of results. Pixel intensity was quantified using Array Vision software. All targets are phospho-specific unless otherwise stated.

Available Kits

Cat.#	Name	Size	Species
#9474	PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout)	32 assays	H, M
#9700	PathScan® Akt Signaling Antibody Array Kit (Fluorescent Readout)	32 assays	H, M
#13047	PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent Readout)	32 assays	H
#13124	PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout)	32 assays	H
#12622	PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout)	32 assays	H
#12785	PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout)	32 assays	H
#7323	PathScan® Intracellular Signaling Array Kit (Chemiluminescent Readout)	32 assays	H, M
#7744	PathScan® Intracellular Signaling Array Kit (Fluorescent Readout)	32 assays	H, M
#7982	PathScan® RTK Signaling Antibody Array Kit (Chemiluminescent Readout)	16 assays	H
#7949	PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout)	16 assays	H
#12856	PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Chemiluminescent Readout)	32 assays	H, M
#12923	PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout)	32 assays	H, M

Species cross-reactivity: H = human, M = mouse.
All kits are available in a carrier-free formulation.

- Kits are available for both chemiluminescent and fluorescent detection.
- Arrays are produced and optimized in-house at CST with the highest quality antibodies, providing you with the greatest possible specificity and sensitivity.
- Technical Support is provided by CST's in-house Molecular Assay Group who develops and produces the products and knows them best.

Visit **www.cellsignal.com/abarray** for additional information.

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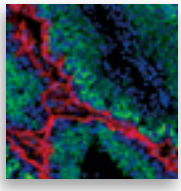

FRONT COVER IMAGE: Vimentin (D21H3) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) #9856 (blue pseudocolor). Miwi (D92B7) XP® Rabbit mAb #6915 (red pseudocolor). Actin filaments were labeled using DY-554 phalloidin (green pseudocolor).

Hongying, Research Associate,
has been with CST since 2006.

FOUNDED BY RESEARCH SCIENTISTS IN 1999, Cell Signaling Technology (CST) is a private, family-owned company with over 400 employees worldwide. Active in the field of applied systems biology research, particularly as it relates to cancer, CST understands the importance of using antibodies with high levels of specificity and lot-to-lot consistency. It's why we produce all of our antibodies in house, and perform painstaking validations for multiple applications. And the same CST scientists who produce our antibodies also provide technical support for customers, helping them design experiments, troubleshoot, and achieve reliable results. We do this because that's what we'd want if we were in the lab. Because, actually, we are.

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