Antibody Specificity
Does your antibody measure up?

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
PD-L1 (E1L3N™) XP® Rabbit mAb
from Cell Signaling Technology

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 consistency, robust results in multiple applications including flow cytometry.

Reactivity: H

Western blot analysis of extracts from A. COS cells (PD-L1 negative) transfected with PD-L1, B. COS cells made interleukin 4 (IL-4) dependent, C. COS cells made interleukin 4 (IL-4) dependent and transfected with PD-L2, and D. COS cells made interleukin 4 (IL-4) dependent and transfected with PD-L1 (positive) using the indicated antibodies.

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/tumorimmune

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 Numerous research manuscripts describing novel biomarkers have reportedly been withdrawn from publication due to mistaken conclusions based on assumed antibody specificity and inadequate controls (1).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

PD-L1

Flow cytometric analysis of untreated COS-MC cells using #13684 (blue) compared to concentration matched Rabbit IgG (DA1E) mAb IgG XP® Isotype Control #3900 (red).

References

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

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


Order your complimentary poster: www.cellsignal.com/tumorimmuno
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

**STEP 2**
Antigen Design & Clonal Expression

900+ clones screened for signal intensity by ELISA

**STEP 3**
Screening for Target Specificity

71 clones screened by western blot

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

- Western Blotting
- Immunoprecipitation
- Immunohistochemistry
- Immunofluorescence
- Flow Cytometry
- 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

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

Dr. Jing Li, PhD
CST Development Sr. Scientist
Joined CST in 1999

140 years of combined experience

CST’s target selection committee

EMT

tight-junction,
adherens-junction,
and desmosome
dissociation

Normal epithelial cells

Carcinoma

Invasive carcinoma

Cell junctions

Desmosomes

Basement membrane

bottom layer

middle layer

top layer

This 3-D molecular model of vimentin can be viewed on Sketchfab.com
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 (tyr1245/1244) (DO1) XP® Rabbit mAb #3077: Comparison of CST #3077 (top) and another company’s product (Company 2, bottom) on IHC-stained lung (A) shows apparent lack of specific staining for CST antibodies. Western blot analysis of cell lines and biologically relevant samples using #3077 and the Company 2 antibody shows that both antibodies recognize several nonspecific bands in these control experiments (bottom). Both membranes were developed on the same film with the same exposure time (10 sec).

In contrast, the Company 2 phospho-Met antibody 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 recognizes other phosphorylated RTKs, another company’s phospho-Met antibody recognizes other phosphorylated RTKs, or a phospho-Met antibody (D26) XP® Rabbit mAb #4370 demonstrates no cross-reactivity in control extracts treated with growth factors that activate other RTKs or that overexpress various other RTKs or phosphorylated proteins (biologically relevant samples). In contrast, the Company 2 phospho-Met antibody recognized several nonspecific 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.

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 PFKL (DO9) Rabbit mAb #4013 was used to detect PFKL (PKLR) in COS cells, it failed to detect the protein, even though western blot analysis shows the protein. CST’s PFKL (DO9) Rabbit mAb #4013 was used to detect PFKL (PKLR) in COS cells, and no signal was detected. CST’s new PFKL (DO9) Rabbit mAb #4013 was used to detect PFKL (PKLR) in COS cells, and a strong signal was detected. CST’s PFKL (DO9) Rabbit mAb #4013 was used to detect PFKL (PKLR) in COS cells, and a strong signal was detected. CST’s PFKL (DO9) Rabbit mAb #4013 was used to detect PFKL (PKLR) in COS cells, and a strong signal was detected.

COS/FGFR1

A431 - HGF

Cell Lines & Treatments

Tyrosine Kinase Cross-reactivity Panel

Cell Lines & Treatments

A

1:200 Dilution

B

1:1000 Dilution

C

1:400 Dilution

D

1:800 Dilution

E

1:200 Dilution

F

1:400 Dilution

G

1:200 Dilution

H

1:400 Dilution

I

1:200 Dilution

J

1:400 Dilution

K

1:200 Dilution

L

1:800 Dilution

M

1:200 Dilution

N

1:400 Dilution

O

1:200 Dilution

P

1:400 Dilution

Q

1:800 Dilution

R

1:200 Dilution

S

1:400 Dilution

T

1:800 Dilution

U

1:200 Dilution

V

1:400 Dilution

W

1:800 Dilution

X

1:800 Dilution

Y

1:200 Dilution

Z

1:400 Dilution

AA

1:800 Dilution

BB

1:200 Dilution

CC

1:400 Dilution

DD

1:800 Dilution

EE

1:200 Dilution

FF

1:400 Dilution

GG

1:800 Dilution

HH

1:200 Dilution

II

1:400 Dilution

JJ

1:800 Dilution

KK

1:200 Dilution

LL

1:400 Dilution

MM

1:800 Dilution

NN

1:200 Dilution

OO

1:400 Dilution

PP

1:800 Dilution

QQ

1:200 Dilution

RR

1:400 Dilution

SS

1:800 Dilution

TT

1:200 Dilution

UU

1:400 Dilution

VV

1:800 Dilution

WW

1:200 Dilution

XX

1:400 Dilution

YY

1:800 Dilution

ZZ

1:400 Dilution

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.

Antigen Receptor (DO111) XP® Rabbit mAb #3077: IC analysis of HCC827 xenograft sections of paraffin-embedded human prostate cancer tissue using #3077 and various IHC reagents, as indicated.

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. This 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.
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

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

siRNA knockdown to test specificity

The accuracy of your immunoassay results is dependent on the quality of the primary antibody used.
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 supported by optimized protocols?

Ensure accurate results in your lab by using protocols optimized to work with CST™ antibodies and your targets. FoxP3 (D06IRB) XP® Rabbit mAb #12653 and FoxP3 (D06IC) 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.

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.

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

Btk is selectively expressed in B cells.

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) #9512: Flow cytometric analysis of fixed and permeabilized cells treated with imatinib compared to untreated controls. Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. Flow cytometric analysis of HeLa cells using multiple lots of #9512 clearly demonstrates a decrease in phospho-Histone H3 (Ser10) content in imatinib-treated cells compared to controls.
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 all 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, must be optimized to work together, especially when the target is highly specific for the target of interest.

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.

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:

1. **Chromatin Integrity**
   - Enzymatic digestion gently fragments the chromatin, protecting the integrity of the protein and the DNA.

2. **Assay Reliability**
   - The Histone H3 antibody is a universal control for tracking assay efficiency and reagent performance.

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

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.
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 modified 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 many biological systems and species to support diverse research interests.
- Expert technical service is provided by CST proteomics scientists throughout your experiment.

Available Kits

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Note: For all assay kits, there is a good second degeneration after the Stage2 purification of enriched peptides (see the protocol after Stage2 Purification.)

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

PathScan® Antibody Array Kits

Multiplex Format

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

Available Kits

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Species cross-reactivity: H = human, M = mouse.

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Species cross-reactivity: H = human, M = mouse.

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

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