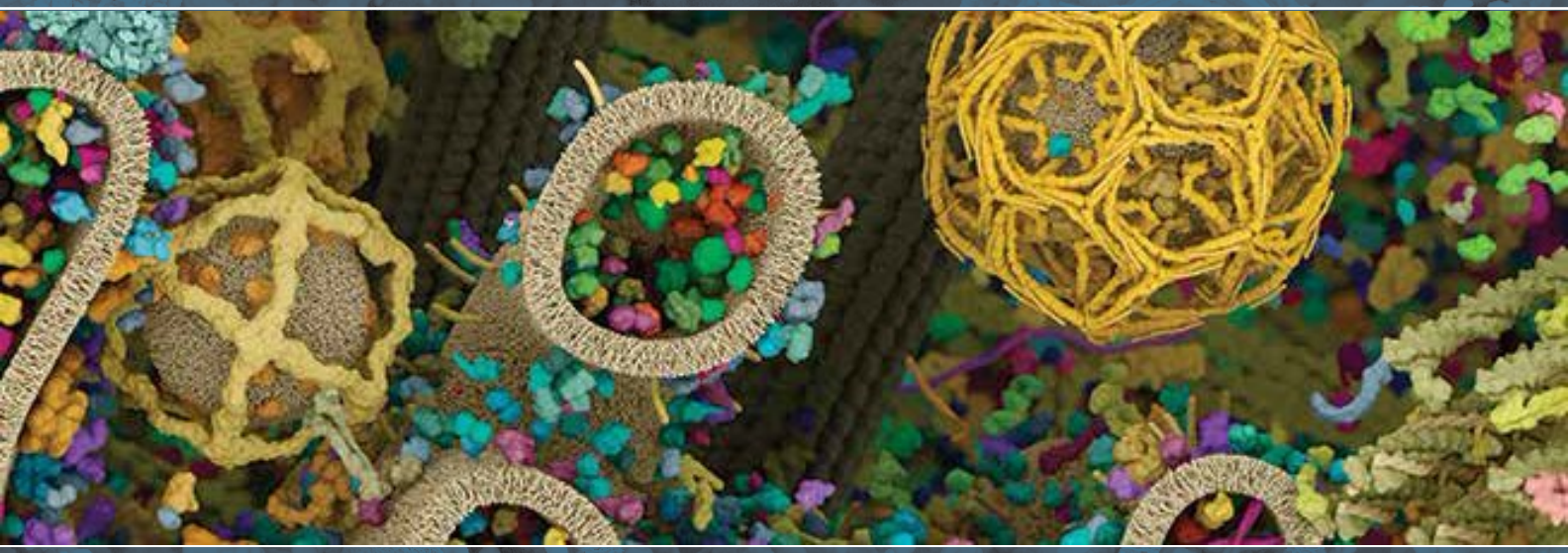
20 Years of Antibody Validation at CST



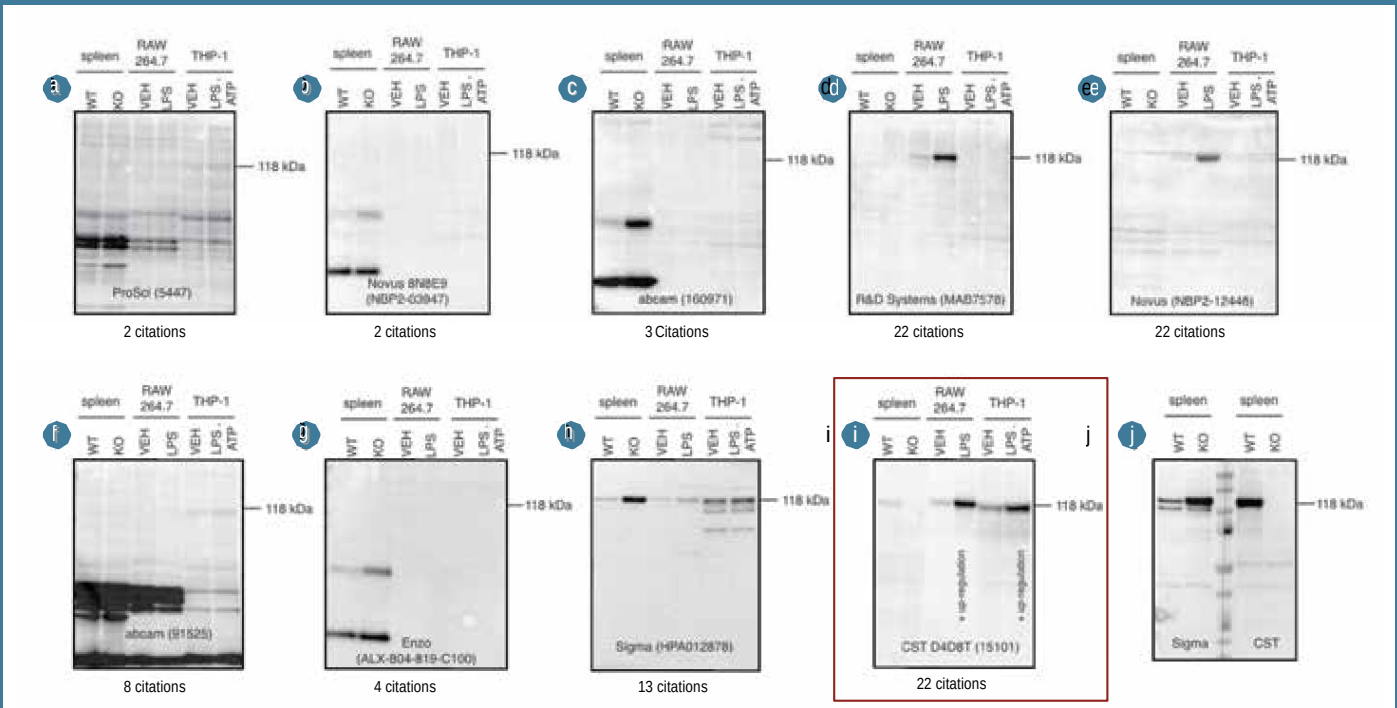
20 THANK YOU 1999-2019

Cell Signaling TECHNOLOGY®

An Example of Using Unreliable, Poorly Validated Antibodies

A recent publication in *Scientifi Reports* rigorously tested 9 commercially available antibodies and found that only one, from Cell Signaling Technology(CST), met all validation criteria. All of the tested antibodies were used in independently published research, and many presented contradictory conclusions about the mechanisms of macular degeneration.

Independent validation of the specificity of commercially available anti-NLRP3 antibodies by western blotting



	Manufacturer	Reactivity	Host	[antibody]	Catalogue #	Lot #
anti-NLRP3	Cell Signaling Technologies	mouse, human	rabbit	WB1:1000	D4D8T,15101	3
	ProSci	mouse, human	rabbit	WB1:1000	5447	6769-1204
	Novus Biologicals	mouse, human	mouse	WB1:1000	8N8E9, NBP2-03947	-
	Novus Biologicals	mouse, human	rabbit	WB 1:400	NBP2-12446	080639650-14
	abcam	human	mouse	WB1:1000	ab160971	GR228391-15
	R&D Systems	mouse	rat	WB 1:250	MAB7578	-
	abcam	mouse, human	rabbit	WB 1:500	ab91525	GR62279-6
	Enzo Life Sciences	human	mouse	WB 1:100	ALX-804-818-C100	11051424
	Sigma	human	rat	WB1:1000	HPA012878	D106015

 Kosmidou C, Efstathiou NE, Hoang MN, Notomi S, et al. Issues with the Specificity of Immunological Reagents for NLRP3: Implications for age-related macular degeneration. Page 4-7. Sci Rep. 2018;8(1):461.

Validating the specifi of commercially available anti-NLRP3 antibodies by western blotting.

Nine commercially available anti-NLRP3 antibodies were tested in terms of their specifi against murine and human positive controls, including mouse spleen tissue, murine RAW 264.7 and human THP-1 macrophage cell lines. Antibody specifi was validated by testing protein expression in spleen tissue from Nlrp3 knockout mice as a negative control. RAW 264.7 cells were primed with (LPS 10 ng/mL) for 6 hours and were compared to vehicle untreated cells. THP-1 macrophages were primed with LPS (10 µg/mL) plus ATP (5 mM) for 3 hours and compared to vehicle control THP-1. 50 µg of total protein were loaded on a gel and blotted with anti-NLRP3 antibodies with the expected molecular weight at ~118 kDa

CST Quality Principles

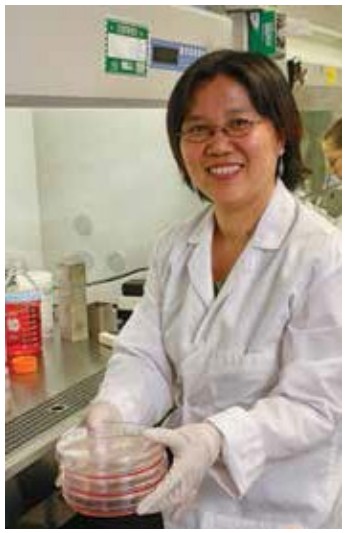
Antibody validation is not a one-size-fits-all process. Our teams of scientists perform custom analysis of each antibody using the most biologically relevant models and assays, and we offer optimized protocols and expert technical support to ensure that our antibodies will work for your research.

☑ Specificity

- **Binary Models:** Analysis of panels of cell lines with high, medium, low, or no protein target expression levels including all relevant controls.
- **PTM-specificity:** Post-translational modification confirmation using appropriate kinase-specific activators, inhibitors, and other treatments (phosphatase, acetylase, PNGase, etc.). Peptide arrays transient expression of site-specific mutants to confirm site-specificity and effects of nearby PTM's on antibody specificity.
- **Genetic Inactivation:** Specificity confirmed with siRNA knockdown, knockout models, and other tools
- **Biologically Relevant Treatments:** Testing cell lines treated with growth factors, cytokines, or chemical activators/inhibitors to knowingly modify target expression, localization, posttranslational modification, etc.
- **On-and-Off Target Binding:** Analysis of multiple normal and diseased tissues to assess performance in a broad spectrum of tissues.

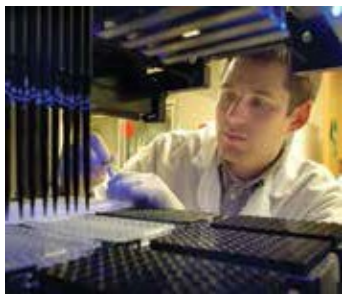
☑ Sensitivity

- Detection of endogenous protein levels across a wide array of cell- and tissue-based samples to verify reactivity and sensitivity.
- Optimized application validation including testing multiple protocols (buffers, conditions, etc.) and application-specific formulation.
- Titration to determine the optimal working concentration in each assay



☑ Consistency

- Lot-to-lot validation (compare new lot to old lot) to ensure consistent performance.
- Recombinant monoclonal antibody technology allows highly controlled, reproducible production of each lot.
- Annual quality control testing for all products.
- Every new product is tested and verified by at least three different internal teams to ensure performance across multiple samples, users, and experiments.



☑ Methodology

- Product-specific optimized protocols
- Recommendations for optimal dilutions and buffers

☑ Support

- Top-ranked global technical support
- The scientists who validated the antibody will help make sure it works in your lab
- Ongoing product testing and validation in new applications based on customer feedback and recommendations.

Only the Best Survive the CST Validation Process

1. Antigen Design

2. Antibody Development and Validation

Tests performed for each application

IHC	ChIP	Flow	IF	WB/IP	
●	●	●	●	●	Genetic models: knockout-derived tissues/cell lines, CRISPR
●	●	●	●	●	Endogenous binary models: positive vs negative expressing cells/tissues
●	●	●	●	●	Knock-down strategies, siRNA/shRNA
●	●	●	●	●	Testing across multiple tissues or cell lines with varied expression levels
●	●	●	●	●	Independent antibody verification using antibodies against the same target but a different antigen
●	●	●	●	●	Comparison to published data
●	●	●	●	●	Controls: Isotype/secondary, loading, quality, etc.
●	●	●	●	●	Multiple rounds of independent testing to verify consistency and reproducibility
●	●	●	●	●	Overexpression of target protein
●		●	●	●	Tissue arrays and rodent tissues
	●	●	●	●	Primary cell lines/differentiated stem cells
●	●		●	●	Treatment induced up- or down-regulation of expression, PTM's and/or changes in localization
●		●	●	●	Phosphatase treatment
●			●	●	Animal models of disease
●			●	●	Cell-cycle-dependent expression, localization
●			●	●	Peptide blocking
●			●		Xenografts (drug-treated and control)
●				●	Human normal and tumor tissues
	●			●	Peptide ELISA, peptide arrays and dot blot
				●	Recombinant proteins
	●				Positive vs. Negative loci
	●				Independent complex subunit verification

Every CST antibody undergoes rigorous application-specific testing customized according to the target and needs of the individual antibody. Listed is a sampling of the methods we employ to ensure our antibodies perform as expected.

We select applications for testing based on the biological function and relevance of the target. During the validation process several antibodies against the same target may be tested - only the best performing, most reproducible antibodies make it in to our catalog.

5
CST Validated Antibody

4
Stability Testing
and
Packaging Decisions

3
Independent QC

Even before we release a product to the scientific community at large, each antibody undergoes even more testing at the hands of our production and application scientists, as well as academic and industry collaborators to ensure each product is specific, functional and optimized for each approved application.