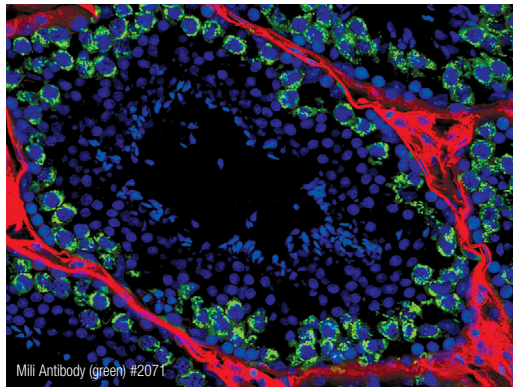
CELL SIGNALING TECHNOLOGY

IF VALIDATED ANTIBODIES

Specificity, consistency and optimized assay conditions are three key elements that help ensure reliable immunofluorescence (IF) staining results each and every time.

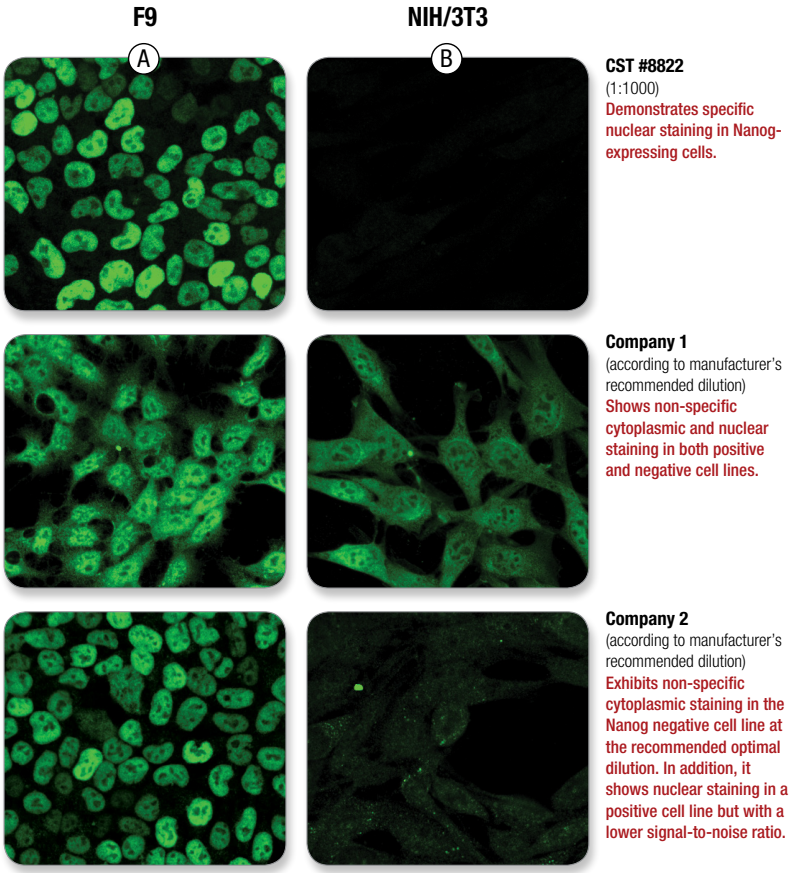


Is your antibody specific?

Detection of a specific band in a western blot does NOT guarantee that the antibody performs specifically for immunofluorescence as well. All CST antibodies approved for use in IF have undergone a rigorous validation process including verification of the correct subcellular localization in target appropriate cell or tissue model systems.

RESULTS:

The specificity of CST antibodies is demonstrated by a robust detection of the target of interest in the appropriate subcellular compartment and the absence of staining in cells devoid of this target.

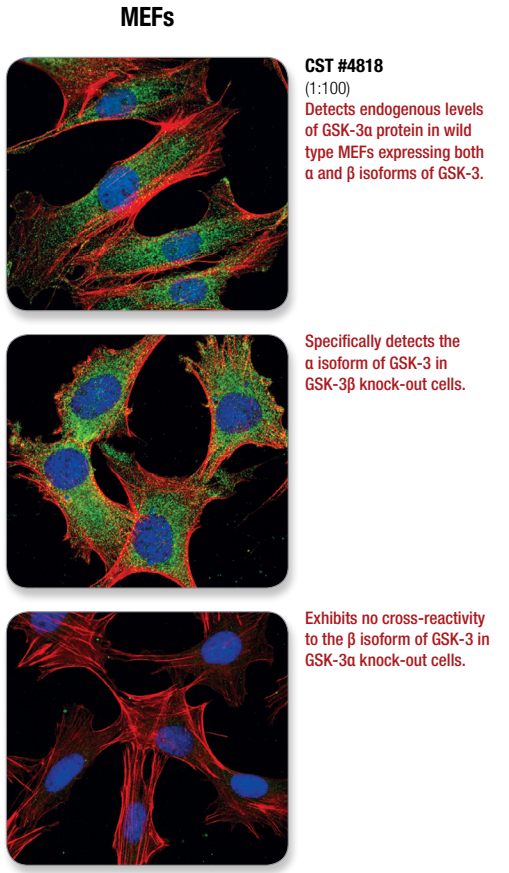


CST #8822
(1:1000)
Demonstrates specific nuclear staining in Nanog-expressing cells.

Company 1
(according to manufacturer's recommended dilution)
Shows non-specific cytoplasmic and nuclear staining in both positive and negative cell lines.

Company 2
(according to manufacturer's recommended dilution)
Exhibits non-specific cytoplasmic staining in the Nanog negative cell line at the recommended optimal dilution. In addition, it shows nuclear staining in a positive cell line but with a lower signal-to-noise ratio.

Nanog (D2A3) XP® Rabbit mAb (mouse specific) #8822: Confocal IF analysis of F9 cells (Nanog positive) **(A)** and NIH/3T3 cells (Nanog negative) **(B)** was performed using #8822 and antibodies from two other companies. All antibodies were used in accordance with manufacturer's recommendations.



CST #4818
(1:100)
Detects endogenous levels of GSK-3α protein in wild type MEFs expressing both α and β isoforms of GSK-3.

Specifically detects the α isoform of GSK-3 in GSK-3β knock-out cells.

Exhibits no cross-reactivity to the β isoform of GSK-3 in GSK-3α knock-out cells.

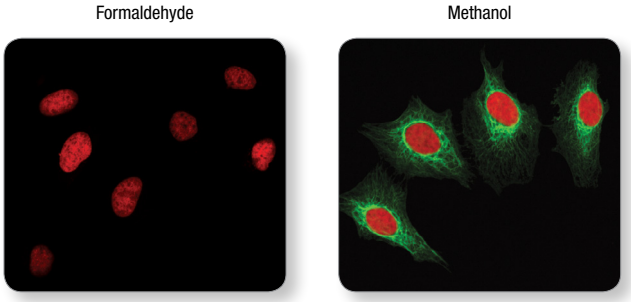
GSK-3α (D80D1) XP® Rabbit mAb #4818. Confocal IF analysis of MEF/GSK-3 wild type cells **(top)**, MEF/GSK-3β (-/-) cells **(middle)** and MEF/GSK-3α (-/-) cells **(bottom)**, using #4818 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). (MEF/wild type, GSK-3α (-/-) and GSK-3β (-/-) cells were kindly provided by Dr. Jim Woodgett, University of Toronto, Canada).

Is your antibody supported by an optimized IF protocol?

Save yourself from spending precious time and reagents on efforts to optimize a protocol that works. At CST, we have determined the fixation, permeabilization, and optimal antibody dilution conditions for you.

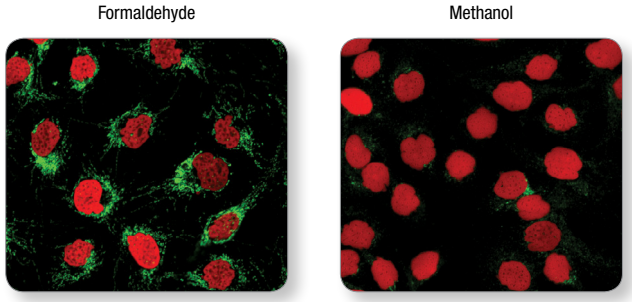
RESULTS:

Optimizing fixation and permeabilization reagents can substantially improve your results.



Keratin 8/18 (C51) Mouse mAb #4546: IF analysis of HeLa cells, fixed with formaldehyde (left) or methanol (right), using #4546 (green). Red = Propidium Iodide (PI)/RNase Staining Solution #4087.

Best with methanol fixation.



AIF (D39D2) XP® Rabbit mAb #5318: IF analysis of HeLa cells, fixed with formaldehyde (left) or methanol (right), using #5318 (green). Red = Propidium Iodide (PI)/RNase Staining Solution #4087.

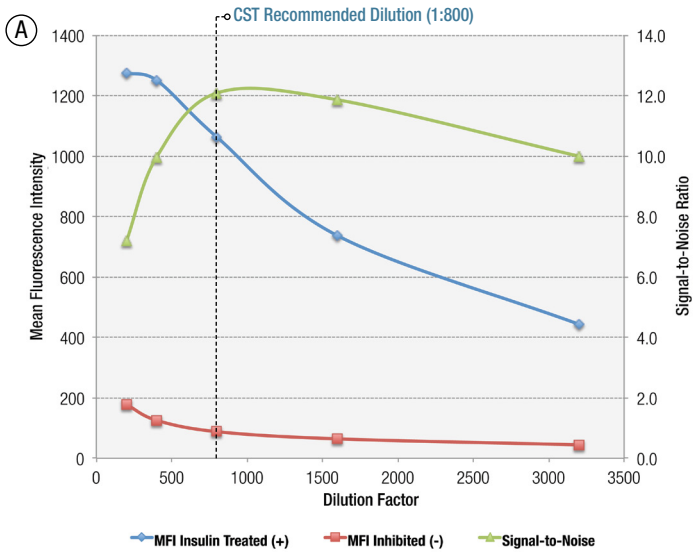
Best with formaldehyde fixation.

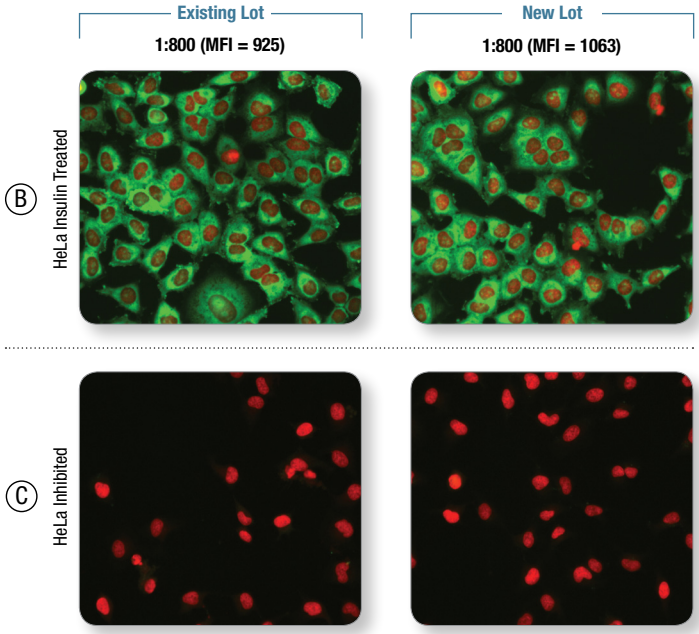
Is your antibody performing consistently?

Ensure reagents are reliable for the life of your project; CST tests every new antibody lot to ensure its performance is equivalent to that of previous lots.

RESULTS:

Stringent testing ensures lot-to-lot consistency.





Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb #5364: Graph depicting mean fluorescence intensity (MFI) and calculated signal-to-noise ratio of HeLa cells serum starved overnight and subsequently insulin treated (100 nM, 30 min) or inhibited with LY94002 #9901 (10 µM , 2 hr), U0126 #9903 (50 µM, 2 hr), and Rapamycin #9904 (100 nM, 2 hr) and stained for IF detection using a new lot of #5364 **(A)**. IF qualitative analysis of HeLa cells either insulin treated **(B)** or inhibited **(C)** at 1:800 dilution using existing and new lots of #5364. Red = Propidium Iodide (PI)/RNase Staining Solution #4087. MFI of the two lots at 1:800 dilution is shown and is comparable, confirming lot-to-lot consistency.

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