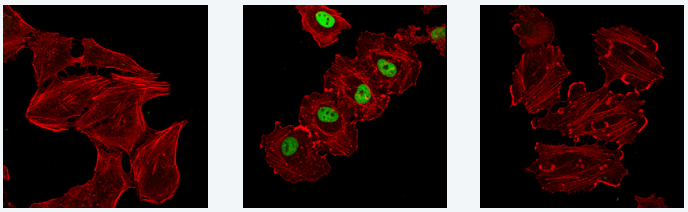
How specific are your IF antibody?

Validating an antibody for immunofluorescence (IF) involves more than just observing a signal in cells. Cell Signaling Technology (CST) thoroughly tests all antibodies recommended for IF for specificity, and establishes optimal assay conditions to help you achieve the best results right from the start.

Verification of Specificity

- Testing multiple cell lines or tissues with known target expression levels
- Verifying subcellular localization
- Subjecting cells to phosphatase treatment, siRNA knock down or over-expression of the target protein
- Using blocking peptides and knock down models
- Confirming specifity using western blot

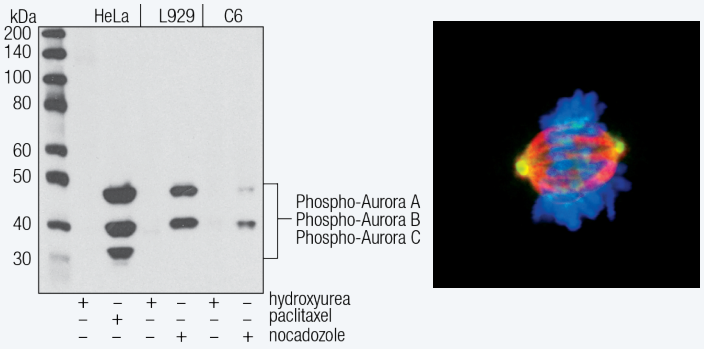
Verification of Phospho-specificity using λ-phosphatase



Starved | TPA | TPA + λ-Phosphatase

TPA treated cells (middle) induce the expression of phospho-c-Fos (green). After treatment with λ-phosphatase the signal disappears proving the specificity of the phospho-c-Fos (Ser32) (D82C12) XP® Rabbit mAb #5348.
Red= DY-554 phalloidin (actin filaments)

Verification of IF Staining Specificity by Western Blot

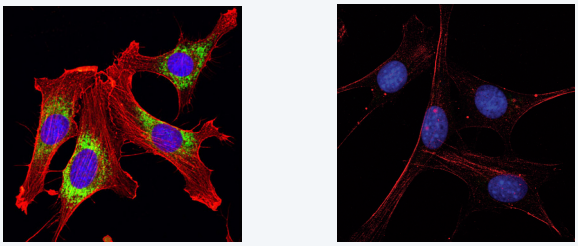


WB analysis on various cell lines treated with hydroxyurea to induce G1/S phase or treated with paclitacel or nocodazole to induce G2/M phase demonstrate that the phosphorylated aurora proteins are only present during G2/M phase of cell cycle. IF analysis of HT-1080 cells using Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) (D13A11) XP® Rabbit mAb #2914 (green) shows that aurora proteins are associated with the mitotic structure.

Protocol Optimization

- Determination of optimal signal-to-noise ratios
- Antibody dilution for fixation and permeabilization, and antigen retrieval conditions are predetermined and specified

Optimization of Permeabilization Conditions

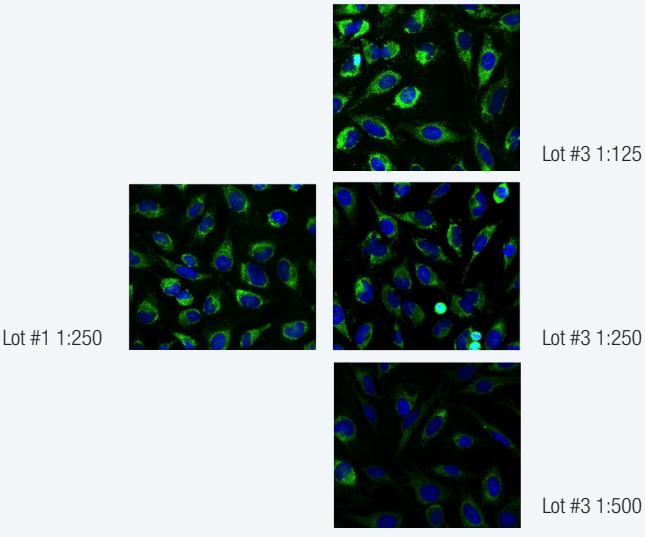


Methanol | Triton X-100

Immunofluorescent analysis of NIH/3T3 cells using PDI antibody #2446 (green) and β-Actin (BH10D10) #3700 (red). NIH/3T3 cells permeabilized with methanol show optimal staining while permeabilized with Triton X-100 shows very weak to no staining. Blue pseudocolor = DRAQ5® #4084 (nuclei).

Lot-to-lot Reproducibility

Comparison of Lot #3 with Lot #1



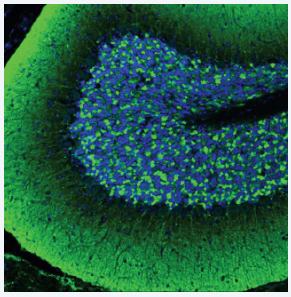
Immunofluorescent titration analysis of HeLa cells using COX IV (3E11) Rabbit mAb #4850 shows the optimal antibody dilution remains 1:250 from lot to lot.
Blue = DRAQ5® #4084 (nuclei).

Conjugated Antibodies for IF Imaging

Cell Signaling Technology conjugates Alexa Fluor® dyes with our highest quality antibodies to give you the brightest signal and lowest background. Conjugation procedures are carefully adapted in-house for each antibody to yield the best fluorescent imaging.

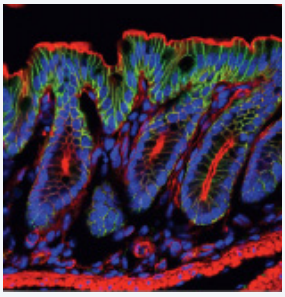
Alexa Fluor® Dyes

- Alexa Fluor® 488, 555, 594, 647 dyes
- Brighter fluorescence output than similar fluorochromes, e.g. FITC, Cy3, Texas Red®-X or Cy5
- Highly photostable
- Spectrum covered by most fluorescence microscopes



CST Conjugated Antibodies

- Optimized in-house conjugation procedure for each antibody
- Tested and validated for fluorescent imaging research
- Primary and secondary conjugated antibodies available
- Convenience of one-step staining
- Alexa Fluor® conjugated secondary antibodies are F (ab')$_2$ fragments, which exhibit reduced background staining



XP® Monoclonal Antibodies – Top Targets for IF

Number	Name	Validated Applications	Species
#4060	Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	W IP IHC-P IHC-F IF-IC F	H M R Hm Mk Dm Z B (C) (X) (Dg) (Pg)
#8768	Stat3α (D1A5) XP® Rabbit mAb	W IP IHC-P IF-IC ChIP	H M R Hm Mk (Pg)
#5018	Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb (Alexa Fluor® 488 conjugate)	IF-IC F	H M R Mk
#12741	LC3A/B (D3U4C) XP® Rabbit mAb	W IHC-P IF-IC	H M R (Mk) (X) (B) (Dg) (Pg)
#4370	Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	W IP IHC-P IF-IC F	H M R Hm Mk Mi Dm Z B Dg Pg Sc (C) (Ce)
#2914	Phospho-Aurora A (Thr288)/Aurora B (Thr232)/ Aurora C (Thr198) D13A11) XP® Rabbit mAb	W IF-IC F	H M R