

Phospho-Specific Rabbit Monoclonal Antibodies Validated for Immunohistochemistry

Cynthia Shapiro, Angela Lightbown, Kathleen Rogers, Jessica Simendinger, Michael Lewis and Katherine Crosby • Cell Signaling Technology, Inc., 3 Trask Lane Danvers, MA 01923

Introduction

Phosphorylation-specific antibodies play an important role in the understanding of cell signaling, cancer biology and in the development of cancer therapeutics. With the implementation of improved methodologies for the development of rabbit monoclonal antibodies, there are now more tools than ever to monitor and measure protein activity. The combined power of phospho-specific antibodies and immunohistochemical analysis provides the clinician, pathologist and researcher tools for identifying the molecular mechanisms underlying a given disease. In this study, a variety of approaches were used to validate antibodies such as P-Akt, P-Erk, P-EGFR, and P-S6 in the immunohistochemical analysis of paraffin-embedded samples. For phospho-specific antibodies, validation methods include the use of untreated and treated cell pellets, peptide blocking, λ phosphatase treatment, mouse models of cancer and drug-treated xenografts.

Methods

Cells were cultured then treated as detailed below:

- LY294002: 50 μM, 2 hrs.
- PMA: 200 nM, 30 min.
- EGF: 100 ng/ml, 5 min.
- Rapamycin: 10 nM, 4 hrs.

After treatment cells were harvested, fixed in 10% NBF for 30 min., washed, combined with Histogel™ then fixed overnight in 10% NBF. Pellets were stored in 70% ethanol before processing by standard methods. Upon cell harvest a small aliquot was removed for the preparation of cell lysate for Western blot analysis, which was performed per standard CST procedure.

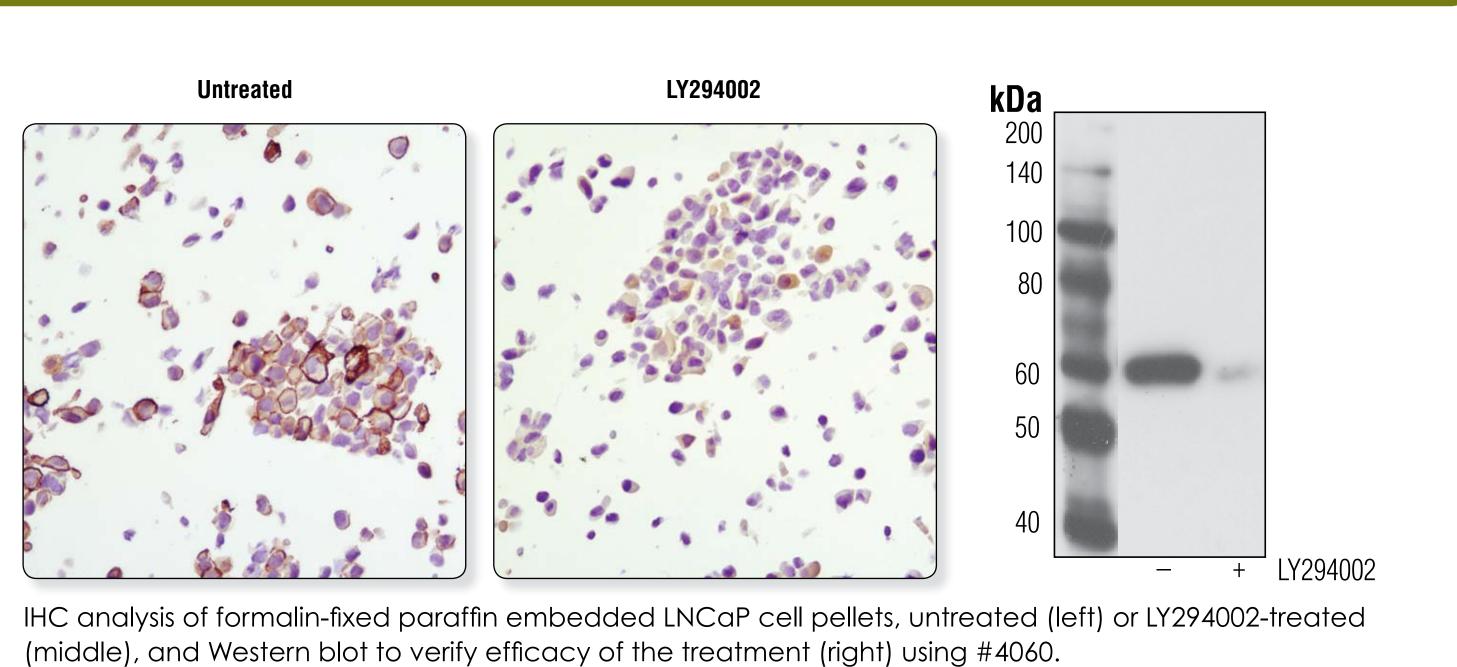
Xenografts were generated using 5-10 million cells in 50% Matrigel injected subcutaneously into Ncr nude mice (Taconic). Mice harboring xenografts were treated with vehicle or as described below:

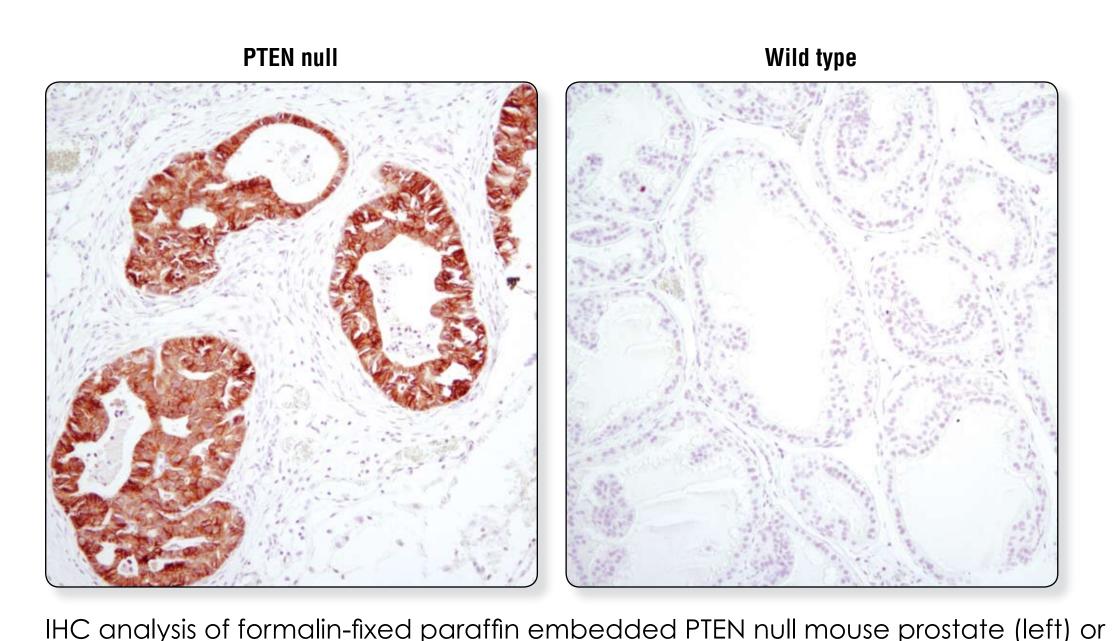
- Gesitinib: 150 mg/kg in Tween-80; harvested 24 hrs. post injection
- Rapamycin: 4 mg/kg in DMSO; harvested 24 hrs. post injection

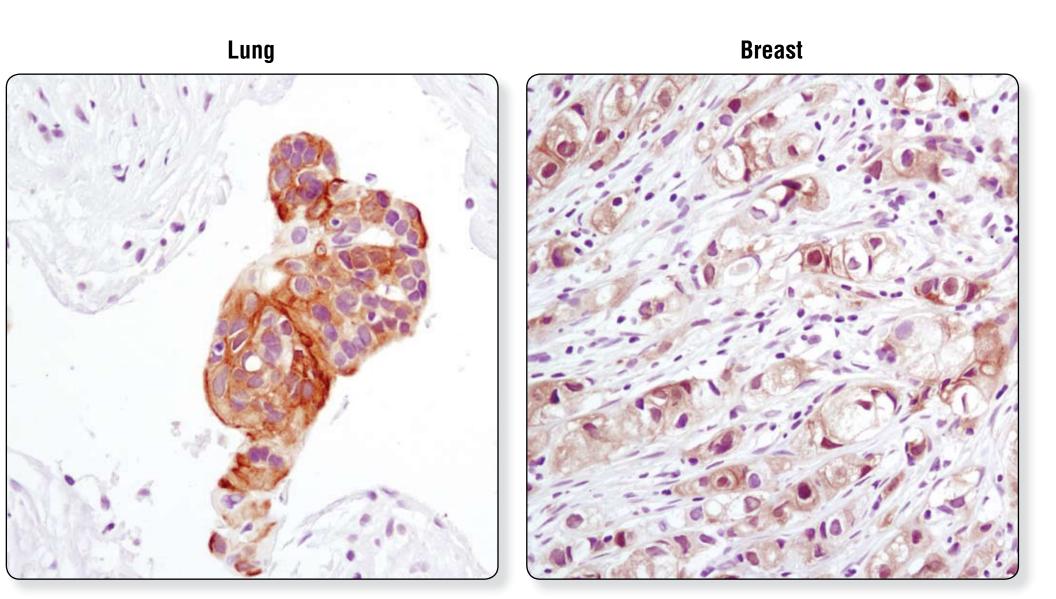
Metastatic tumors were initiated by injecting 2 x 10° cells via the tail vein of Ncr nude mice (Taconic). Lungs were harvested 6 weeks post

Xenografts and lungs were fixed overnight in 10% NBF, then processed per standard methods. All tissues were sectioned at 4 µm and immunohistochemical analysis was performed per standard CST procedure.

Phospho-Akt (Ser473) (D9E) Rabbit mAb #4060

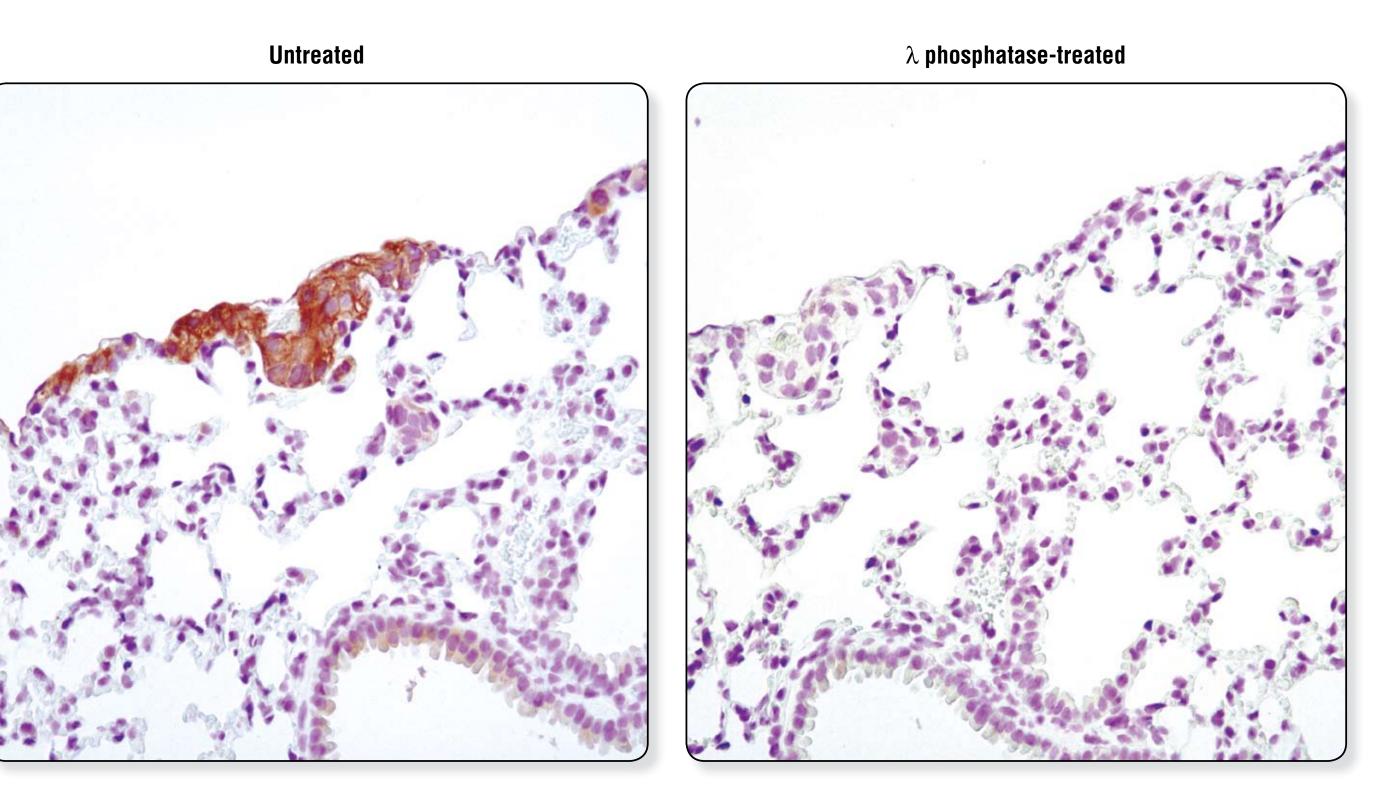






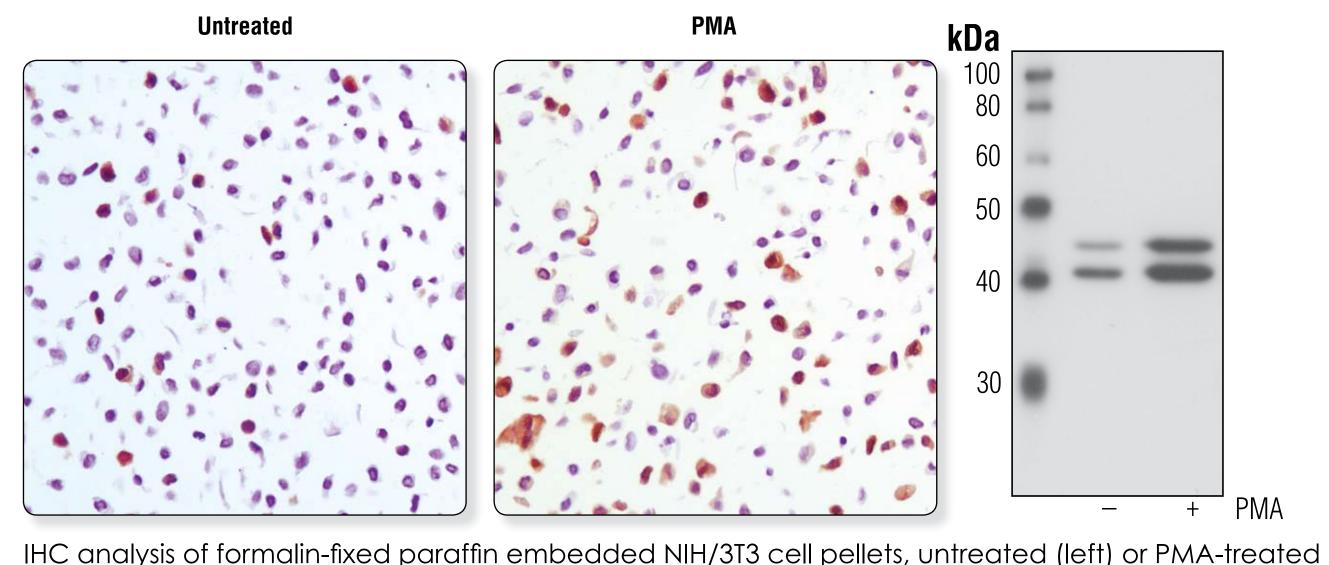
wild type (right) using #4060. (Tissue courtery of David Guertin MIT, Cambridge, MA.)

and breast carcinoma (right) using #4060.

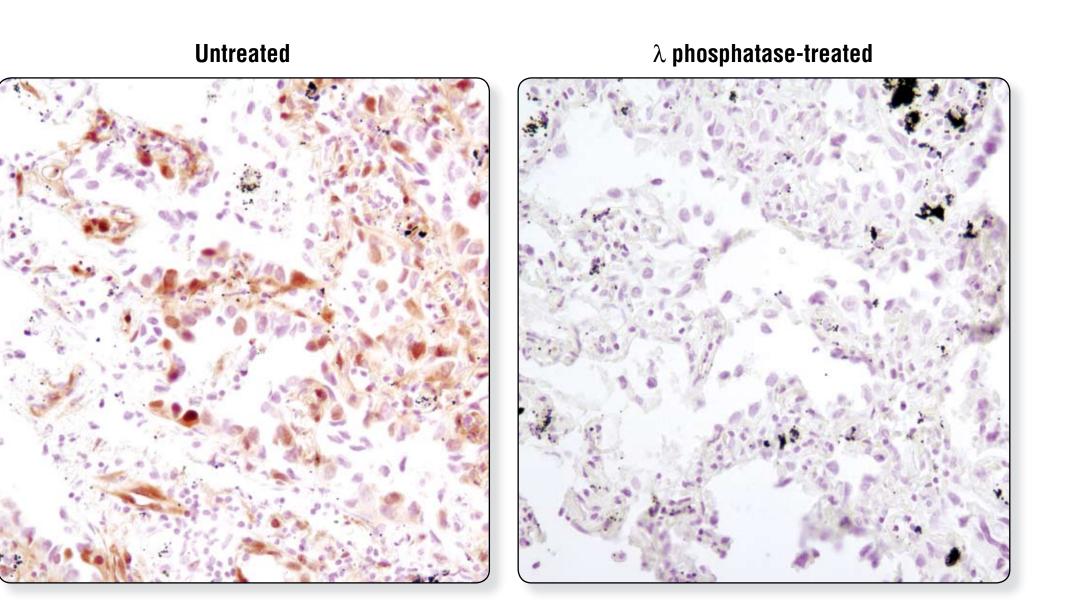


IHC analysis of metastatic SK-OV-3 tumor in mouse lung, untreated (left) or λ phosphatase-treated (right), using #4060.

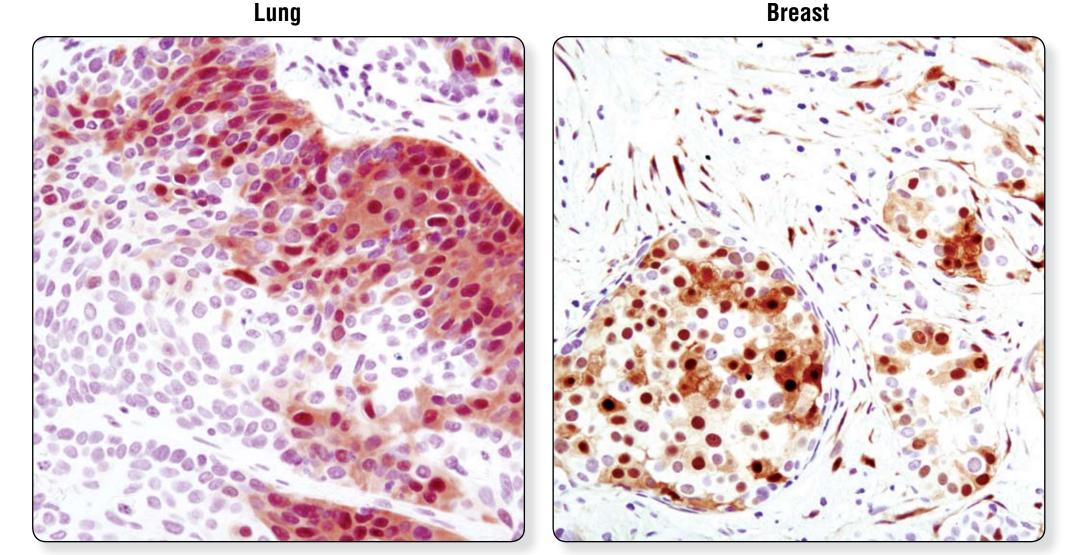
Phospho-p44/42 MAPK (Thr202/Tyr204) (D13.14.4E) Rabbit mAb #4370



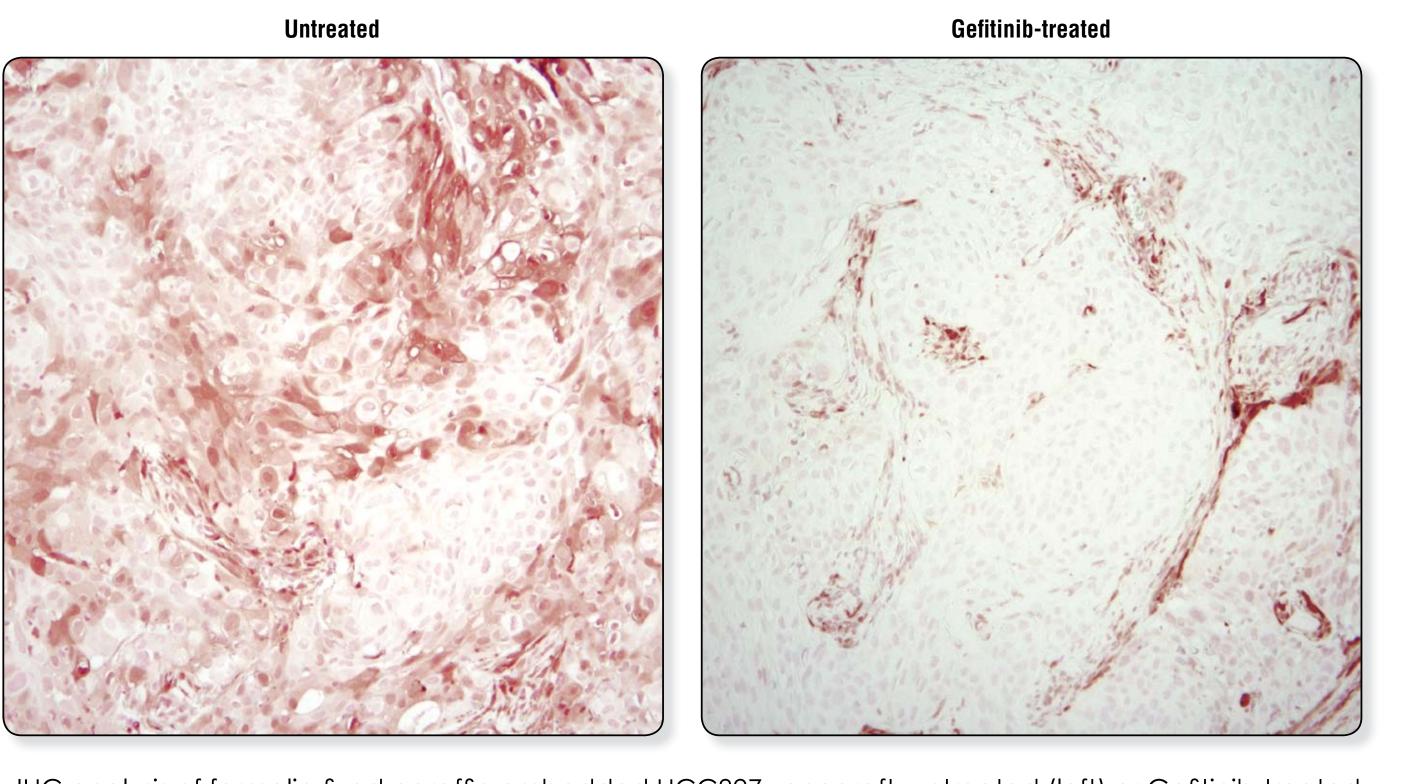
(middle), and Western blot to verify efficacy of the treatment (right) using #4370.



IHC analysis of formalin-fixed paraffin embedded human lung carcinoma, untreated (left) or λ phosphatase-treated (right), using #4370.

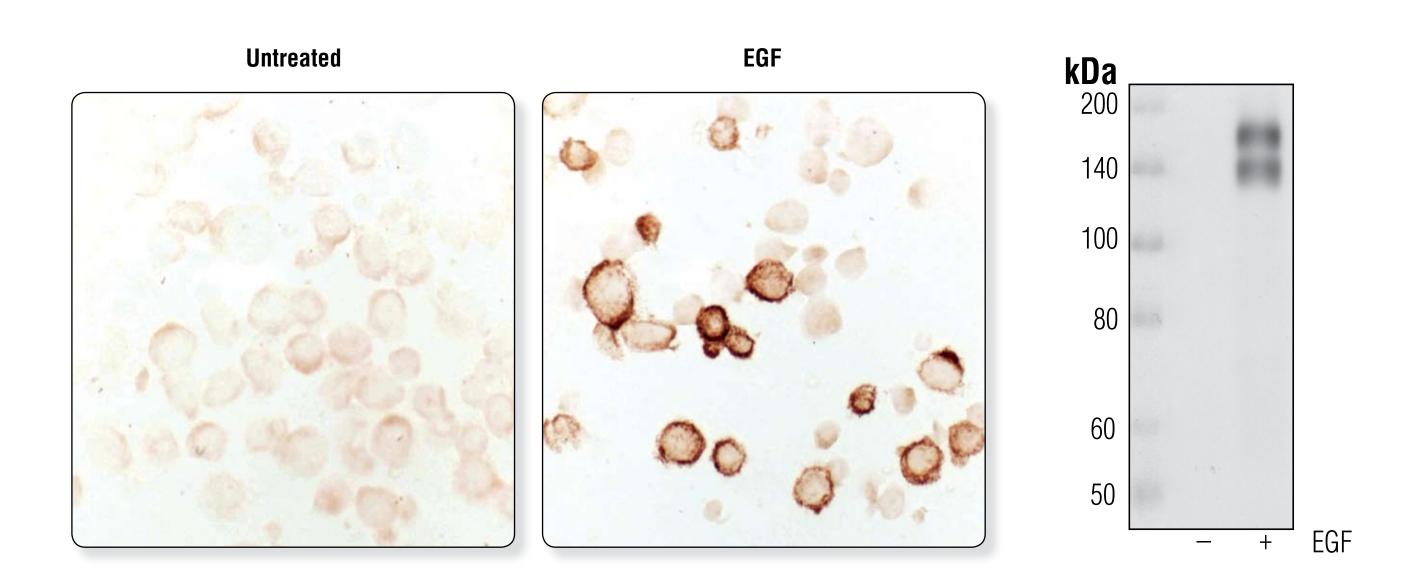


IHC analysis of formalin-fixed paraffin embedded human lung carcinoma (left) and breast carcinoma (right) using #4370.

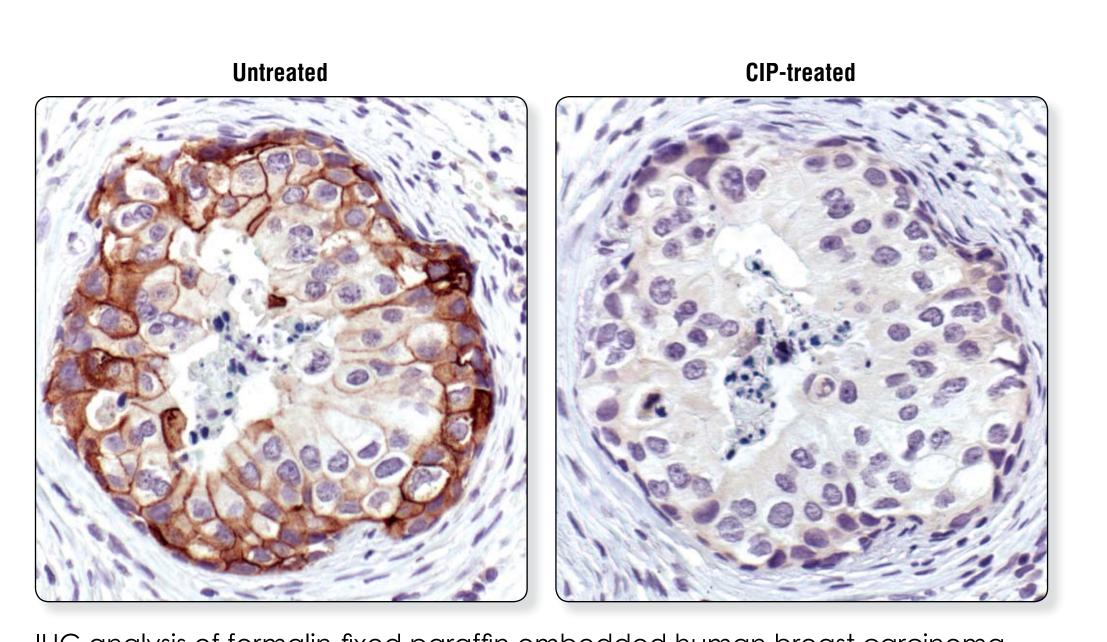


IHC analysis of formalin-fixed paraffin embedded HCC827 xenograft, untreated (left) or Gefitinib-treated (right), using #4370.

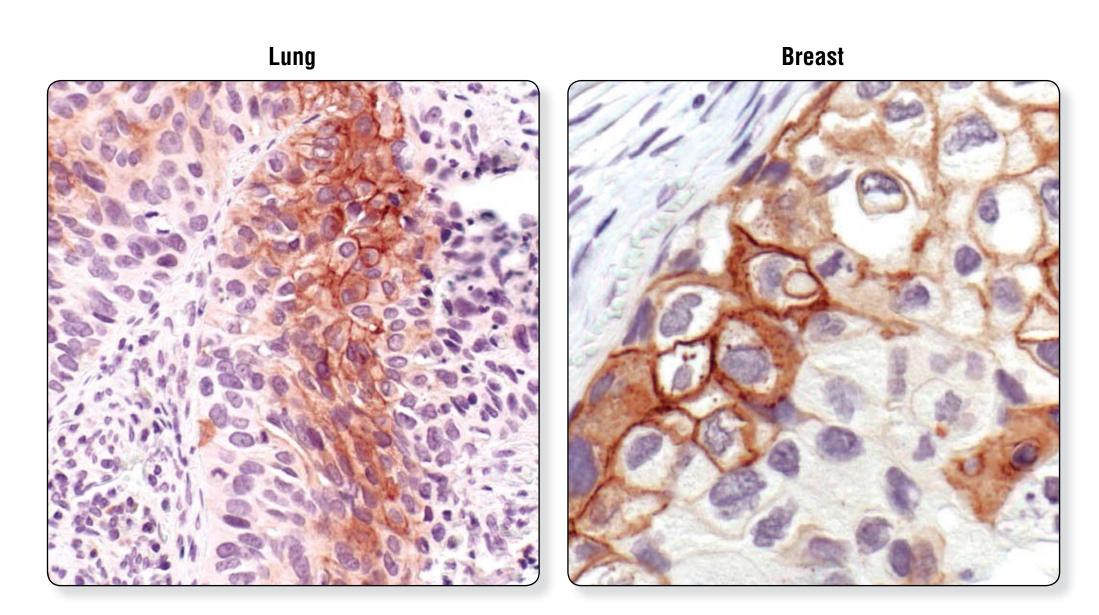
Phospho-EGF Receptor (Tyr1173) (53A5) Rabbit mAb #4407



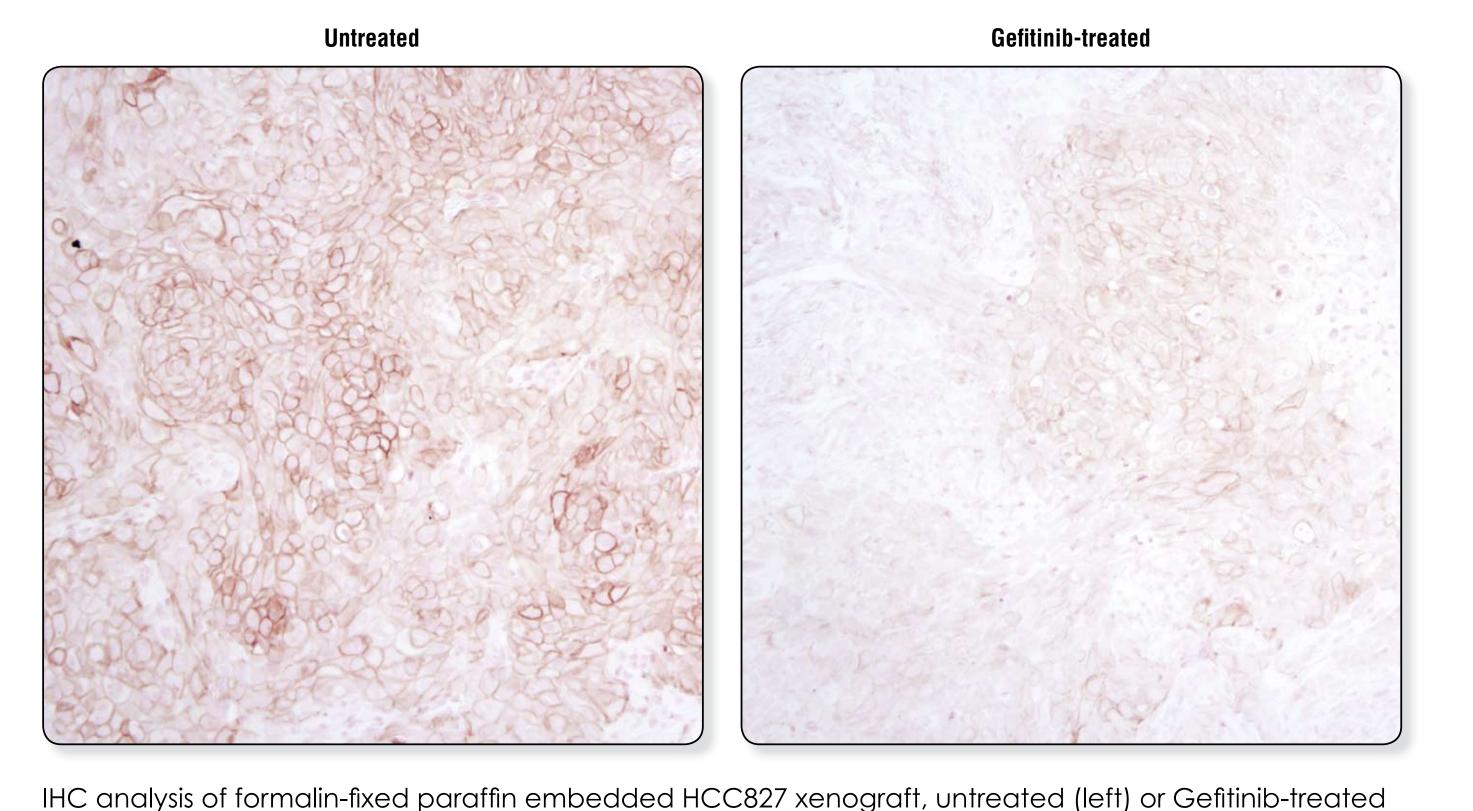
IHC analysis of formalin-fixed paraffin embedded MDA-MB-468 cell pellets, untreated (left) or EGF-treated (middle), and Western blot to verify efficacy of the treatment (right) using #4407.



untreated (left) or Calf Intestinal Phosphatase-treated (right), using #4407.

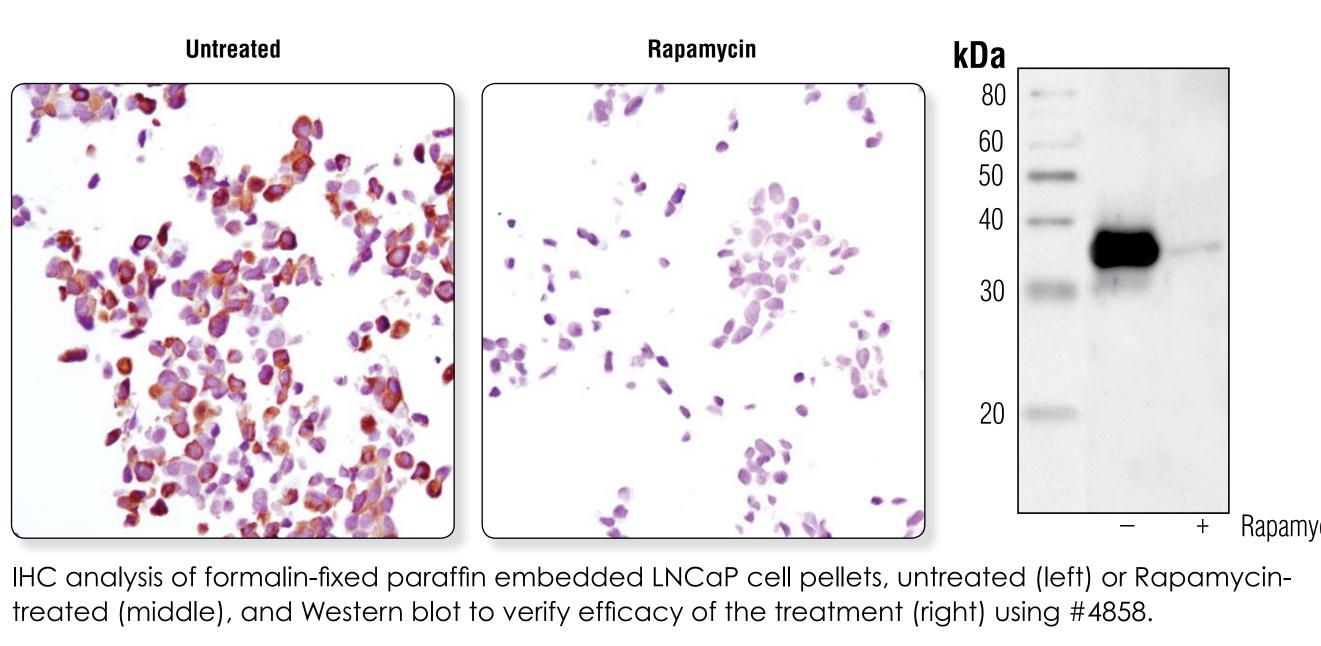


IHC analysis of formalin-fixed paraffin embedded human lung carcinoma (left) and breast carcinoma (right) using #4407.

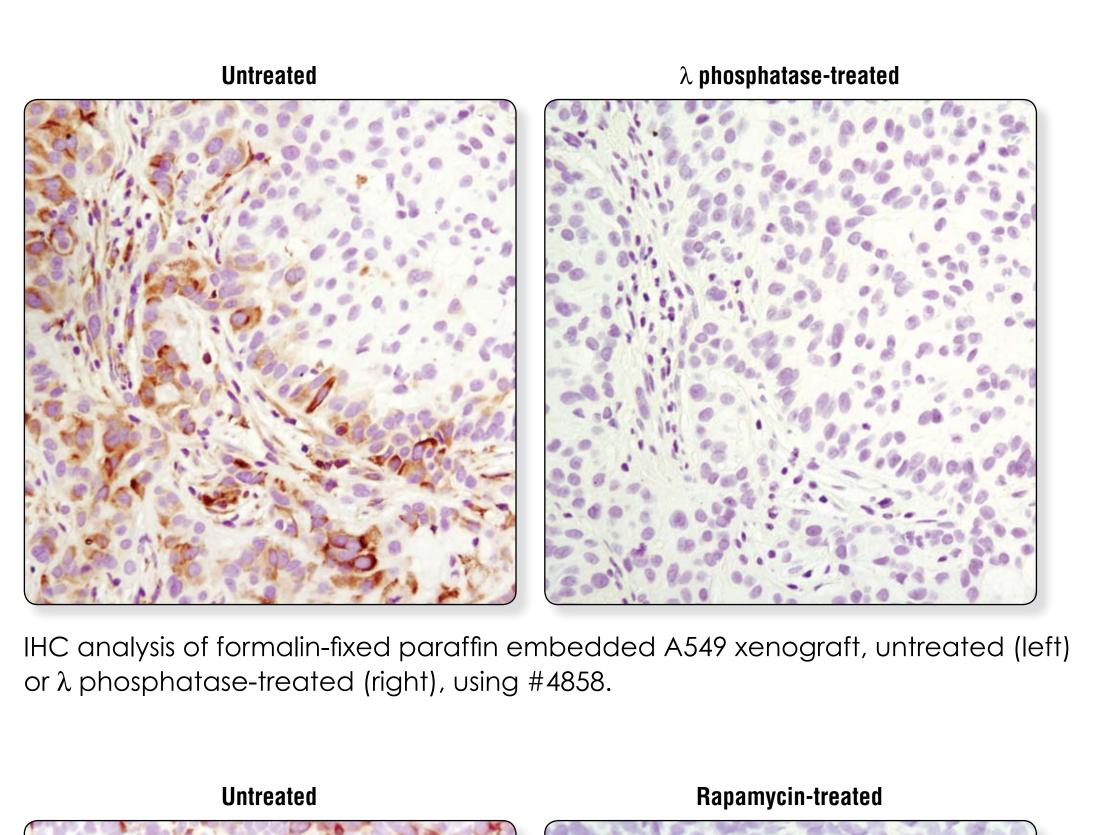


(right), using #4407.

Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) Rabbit mAb #4858



treated (middle), and Western blot to verify efficacy of the treatment (right) using #4858.



IHC analysis of formalin-fixed paraffin embedded Rh30 xenograft, untreated (left) or Rapamycin-treated (right), using #4858.

Conclusion

- The determination of target specificity with phospho-specific antibodies in immunohistochemical analysis requires multiple steps and tools.
- While no one piece of data is sufficient to demonstrate the specificity of staining, collectively these data offer evidence that the staining achieved with a particular antibody is specific.

