Phospho-Specific Rabbit Monoclonal Antibodies Validated for Immunohistochemistry

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Introduction

Phosphorylated proteins act as messengers to transmit signals from extracellular pathways to the nucleus. Proteins commonly implicated in cancer signaling pathways, such as Akt and EGFR, serve as downstream targets of receptor tyrosine kinase activity. The combined power of phospho-specific antibodies and immunohistochemical analysis provides the clinician, pathologist and researcher with a valuable tool for identifying the cellular mechanisms underling a given disease. In this study, a variety of antibodies were used to validate antibodies such as P-Akt, P-Erk, P-EGFR, and P-S6 in the immunohistochemical analysis of formalin-fixed paraffin-embedded tissues.

Methods

Cells were cultured as detailed below:

- 129/WeHI-1B: 35.3% 7d, 10% FBS
- HCT116: 100 mg/ml 5 min
- 492: 10 μM, 4 hrs.
- Rapamycin: 10 μM, 4 hrs.
- Gefitinib: 150 mg/kg in Tween-80, harvested 24 hrs. post injection

After treatment cells were harvested, fixed in 10% MBF for 10 min, washed, combined with DAB™ Plus rapid detection and washed in 10% MBF. Pellets were stained 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.

Immunohistochemical staining, collectively these data offer evidence that the staining achieved in immunohistochemical analysis requires multiple steps and tools.

Conclusion

- 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.

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