Comparison of Commercially Available Phospho-AKT [Ser473] Antibodies for Immunohistochemical Biomarker Analysis

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Abstract

Immunohistochemical (IHC) analysis of AKT phosphorylation on Ser473 is commonly performed on pre- and post-treatment cancer biopsies as part of pharmacodynamic biomarker assessment for targeted oncology therapeutics. The current study was performed to assess the specificity and sensitivity of four pAKT Ser473 monoclonal antibodies for IHC analysis (clones D9E, 736E11, 587F11 and 14-5). To facilitate this analysis, cancer cell lines with constitutive activation of the PI3K pathway due to mutations in PIK3CA (MCF-7 and MKN-1) or PTEN (MDA-MB-468) were grown in the presence or absence of inhibitors of the PI3K pathway (wortmannin and LY294002). Cell lysates were prepared for analysis via Western blotting and formalin-fixed, paraffin-embedded cell pellets were prepared for analysis via IHC. All antibodies produced a band at 65 kDa, the expected molecular weight for pAKT, when used for Western blot analysis of untreated cell lines and demonstrated a reduction or ablation of this band following treatment of the cell lines with inhibitors of the PI3K pathway. When examined via IHC, antibodies 736E11 and 587F11 demonstrated nuclear and cytoplasmic staining in MCF-7 and MKN-1 cells and membrane staining in MDA-MB-468 cells. These antibodies demonstrated a reduction of staining in all three cell lines treated with PI3K inhibitors, but the reduction was modest in MCF-7 and MKN-1 cells. Clone 587F11 was the least sensitive for IHC. Staining with antibodies 14-5 and D9E was predominantly cytoplasmic or membrane associated, while clone14-5 produced a more punctate appearance. Strong staining was achieved with clone 14-5 and prominent reduction or ablation of staining was observed in all three cell lines treated with PI3K pathway inhibitors. Staining with clone D9E was a little less sensitive than with clone 14-5, but significant reduction or ablation of staining in cell lines treated with PI3K inhibitors was also observed. In summary, clones 14-5 and D9E appear to demonstrate adequate specificity and sensitivity for use in IHC assays.

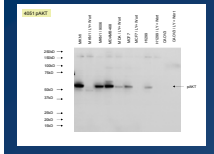
Introduction and Methods

AKT phosphorylation at Ser473 provides a critical regulatory role in diverse cellular processes that are important in oncology, including cell growth, cell cycle, and apoptosis. pAKT immunohistochemistry has been used as a pharmacodynamic biomarker in clinical trials to evaluate the activity of numerous compounds targeting HER2, EGFR, FAK, PI3K, c-MET and other upstream molecules. Some studies have identified reduction in pAKT in response to effective therapy. However, one of the challenges in using phospho-specific antibodies for immunohistochemistry is the concern that cross-reactivity may lead to erroneous conclusions.

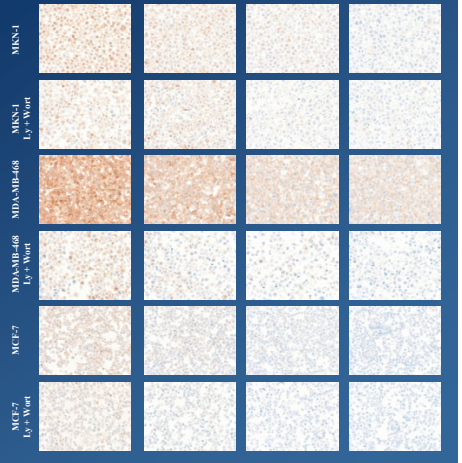
The current study was designed to address the specificity and sensitivity of four leading commercially available pAKT Ser473 monoclonal antibodies (Cell Signaling clone D9E – Cat#4060, Cell Signaling clone 736E11 – Cat #3787, Cell Signaling clone 587F11 – Cat #4051, and DAKO clone 14-5 – Cat #M3628) by comparing Western blot to immunohistochemistry results. Cell lines with constitutive activation of the PI3K pathway due to mutations in PIK3CA (MCF-7 and MKN-1) or PTEN (MDA-MB-468) were grown in the presence or absence of inhibitors of the PI3K pathway (wortmannin and LY294002). Cell lysates were prepared for analysis via Western blotting and formalin-fixed, paraffin-embedded cell pellets were prepared for analysis via IHC. Numerous antigen retrieval conditions were evaluated for each antibody to maximize staining intensity. The primary antibody was titered in an attempt to identify a concentration that demonstrated maximum sensitivity and appropriate reduction in staining in the PI3K inhibitor-treated cells.

Results

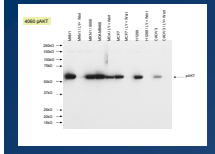
Cell Signaling Clone 587F11 – Cat #4051



Clone 587F11 demonstrated some extra high MW bands by Western blot, lower sensitivity than other antibodies, and incomplete reduction of staining in PI3K-inhibitor treated cells by IHC.

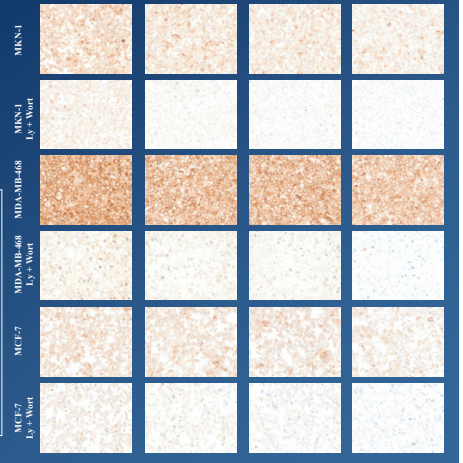


Cell Signaling Clone D9E – Cat #4060

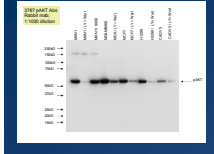


Clone D9E demonstrated a single band consistent with pAKT by Western blot, acceptable sensitivity and reduction of staining in PI3K-inhibitor treated cells by IHC.

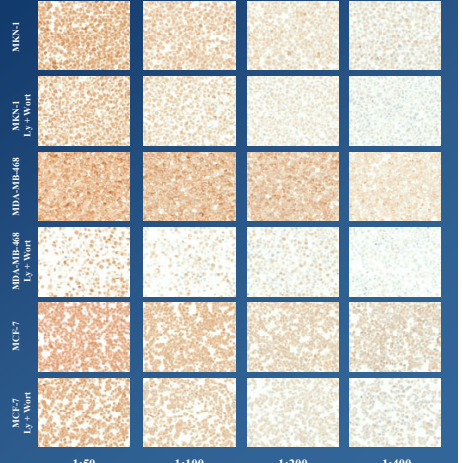
Demonstrated acceptable specificity and sensitivity



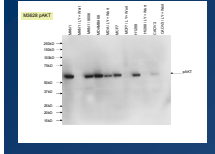
Cell Signaling Clone 736E11 – Cat #3787



Clone 736E11 demonstrated some extra high MW bands in MKN-1 cells by Western blot and poor reduction of staining in PI3K-inhibitor treated MCF-7 and MKN-1 cells by IHC.

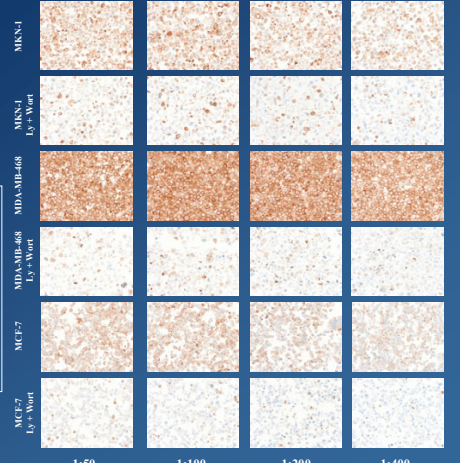


DAKO Clone 14-5 – Cat #M3628



Clone 14-5 demonstrated a single band consistent with pAKT by Western blot, excellent sensitivity and reduction of staining in PI3K-inhibitor treated cells by IHC.

Demonstrated acceptable specificity and sensitivity



Conclusions

- Properly-designed immunohistochemistry assays using clones **14-5** and **D9E** appear to demonstrate appropriate specificity and sensitivity for pharmacodynamic monitoring.
- Testing phospho-epitope specific antibodies for immunohistochemistry using multiple cell lines and signal transduction pathway inhibitors is an important step in assay qualification. Testing only one cell line (e.g. MDA-MB-469) may lead to false confidence in antibody specificity.
- Prior studies using clone 736E11 pAKT antibody for IHC may have under-reported reductions in AKT phosphorylation due to cross-reactivity or non-specific staining. Studies using clone 587F11 for immunohistochemistry may have under-reported expression of pAKT due to reduced sensitivity.