

## p63/P504S Antibody Cocktail in Prostate Biopsies

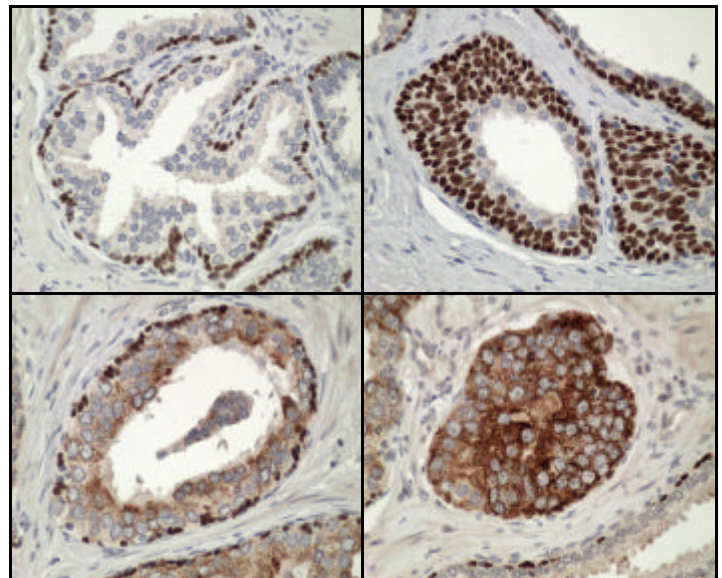
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Antibody cocktails are certainly nothing new in diagnostic immunohistochemistry. However, the combination of an antibody with nuclear reactivity with another antibody with cytoplasmic reactivity is novel. This month we report the utility of such an antibody cocktail in the study of prostate biopsy specimens.

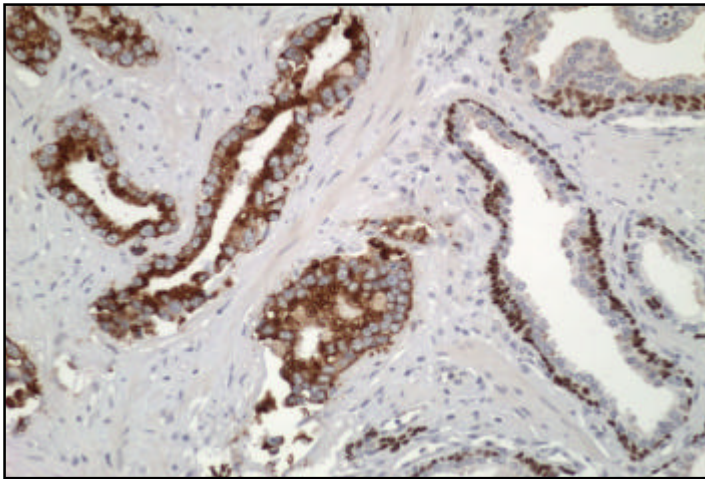
It is well established that stains for prostatic basal cells are extremely useful in the study of prostate biopsy specimens. Although the prostatic basal cell layer may be discontinuous at the periphery of lobules of prostatic acini, prostate adenocarcinoma is characterized by a complete lack of basal cells. Therefore, assuming adequate tissue and good immunostaining technique, the failure to demonstrate prostatic basal cells in an area morphologically suspicious for carcinoma supports a malignant interpretation.

The first marker employed for the purpose of demonstrating prostatic basal cells was high molecular weight cytokeratin, which identifies prostatic basal cells very nicely, and is still in wide use today for this purpose. In the past several years a number of authors have studied the use of antibodies to p63 for detection of prostatic basal cells. P63 is a homologue of the p53 gene, and experimental studies have shown that p63 is necessary for normal breast and prostate development. Unlike the cytoplasmic character of high molecular weight cytokeratin immunoreactivity, p63 stains the nuclei of prostatic basal cells. (Those interested in further details about this antibody may refer to the September 2001 edition of ProPath "Focus on Immunohistochemistry").



*p63/P504S immunostains in prostate tissue. Benign glands (top left) show intact p63-positive basal cells (nuclear staining) with no cytoplasmic P504S reactivity. Basal cell hyperplasia is easy to identify (top right) by extensive p63 staining. As expected, high grade PIN (lower left) demonstrates intact p63-positive basal cells, and also has cytoplasmic P504S. Invasive carcinoma (lower right) stains intensely with P504S but lacks a p63-positive basal layer.*

P504S is a gene that was found to show elevated expression in prostate cancers. This gene codes for a protein identified as  $\alpha$ -methylacyl-CoA-racemase (AMACR) and is located in mitochondria and peroxisomes. As such, antibodies raised to this protein show cytoplasmic reactivity. A number of investigators have shown that antibodies to this overexpressed protein are useful in recognition of prostate carcinoma (see November 2002 issue of the ProPath "Focus on Immunohistochemistry").



*p63/P504S immunostain shows invasive carcinoma (left) with adjacent benign glands (right)*

In late 2002 I was contacted by Dr. David Tacha (of BioCare Medical in Walnut Creek, CA), who came up with the idea of making an antibody cocktail of p63 and P504S. He was seeking a partner to study the utility of this antibody cocktail in prostate biopsy specimens. Shortly after, Dr. Tacha's lab and ProPath embarked on a collaborative study using the reagent that Dr. Tacha prepared. Our work was published last month (March 2004) in *Applied Immunohistochemistry and Molecular Morphology*, and a similar report also appeared in the March 2004 issue of the *American Journal of Clinical Pathology*, authored by Sanderson et al.

We found this antibody cocktail to be particularly well suited to study prostate biopsy specimens. Since p63 stains the nuclei of basal cells and P504S the cytoplasm of prostate carcinoma and high-grade PIN, the reactivity of each antibody is readily apparent, and reactivity with one marker does not "mask" reactivity with the other marker. With this antibody cocktail, one is able to assess both the presence or absence of basal cells and the presence or absence of cytoplasmic reactivity with P504S, using only one immunostain. This can be of particular importance in cases where the atypical areas of interest are so small that only one immunostain can be performed. In cases where the area of interest no longer remains in the paraffin block, immunostaining with this antibody cocktail can be readily performed using tissue transfer techniques or tissue protection techniques (see March 2002 ProPath "Focus on Immunohistochemistry" for details on tissue protection immunohistochemistry). By using these

techniques with the p63/P504S antibody cocktail, there is great potential for decreasing the need for rebiopsy in patients with small atypical lesions on prostate needle biopsy.

p63/P50S antibody cocktail has been available in the ProPath immunohistochemistry laboratory for over a year. In cases where the atypical focus of interest is present only on a previously-stained H&E slide, we are ready and able to provide expert assistance with both tissue transfer or tissue protection immunohistochemistry techniques, depending upon whether the H&E slide is an adhesive slide or a non-adhesive slide.

#### REFERENCES:

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