Immunohistochemistry in Prostate Pathology

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Dr. Kristiansen’s research field covers diagnostic and prognostic biomarkers of solid tumors, with a predominant focus on prostate cancer. He has published more than 270 peer reviewed papers and is a frequent speaker in national and international conferences. In Bonn, he has established next to his translational research working group a GU pathology consult service, is active in postgraduate teaching of pathologists and urologists and is centrally involved in several prostate cancer studies (including the german PREFERE-trial, funded by the German Cancer Aid). He is also part of the steering committee of the European Network of Uropathology (ENUP).

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Dr. Epstein has over 700 publications in the peer-reviewed literature and has authored 49 book chapters. His most-frequently cited first or last authored publications is “Pathological and Clinical Findings to Predict Tumor Extent of Nonpalpable (stage T1c) Prostate Cancer,” published in JAMA, which establishes the criteria for active surveillance. He was also the leading author to develop the WHO Consensus Conference on Classification of Urothelial Neoplasia (1998) and the consensus on updating the Gleason grading system (2005).

He is the author or co-author of five books including “Interpretation of Prostate Biopsies” which is in its 4th edition, and “Bladder Biopsy Interpretation” which is in its 2nd edition. He is a co-editor of the “WHO Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs”, and a co-author of the 2011 AFIP Fascicle, 4th Series on “Tumors of the Prostate Gland, Seminal Vesicles, Male Urethra, and Penis”. He has one of the largest surgical pathology consulting services in the world with approximately 12,000 cases per year, covering the full range of urologic pathology. Dr. Epstein uses these consultations to train four genitourinary pathology fellows each year, with 42 fellows trained to date.
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Prostate Cancer

Prostate cancer is the second most common cancer in men in the United States. It is estimated that about 240,000 new cases of prostate cancer will be diagnosed annually. This accumulates to 16% of all men will be diagnosed with prostate cancer during his lifetime with an average age at the time of diagnosis about 67 years old. Almost 30,000 men will die of prostate cancer in 2013 in the US making it the second leading cause of cancer death in American men, behind only lung cancer (statistics from www.cancer.org).

Immunohistochemical (IHC) markers are often used as an aid in the diagnosis of prostatic adenocarcinoma, especially in the diagnosis of limited primary prostate carcinoma on needle biopsy. The diagnosis of prostate adenocarcinoma is aided by IHC staining for basal cell layer markers, such as p63, cytokeratin 5/6 (CK 5/6), and high molecular weight cytokeratin (CK HMW) as well as prostate-‘specific’ markers.

This document will discuss the potentials and pitfalls of the individual markers used in the diagnosis of prostate cancer.

Adenocarcinoma of the Prostate (Limited)

Negative Markers of Malignancy
- Basal Cell Markers

The loss of basal cells in prostate carcinomas is the most important diagnostic hallmark of malignancy, and basal cell markers has been the immunohistochemical cornerstone of prostate diagnostics for more than 15 years (1,2). Malignancy is strongly supported by the absolute absence of basal cell staining by IHC in a morphologically suspicious lesion. The lack of basal cell layer staining should be supported by the simultaneous demonstration of a positive basal cell layer in adjacent unequivocally benign glands (that serve as an internal quality control). Basal cell cytokeratins (CK HMW, CK 5/6, CK 14) and p63 are both equally eligible for staining of basal cells and yield similar results (Figure 1) (3,4). The sensitivity to detect basal cells can even be increased by a combination of both (5,6).

A lack of basal cell staining may also be seen in several benign mimickers of prostatic adenocarcinoma. In adenosis (atypical adenomatous hyperplasia (AAH)), usually >50% of the glands label with basal cells markers, yet as few as 10% may be positive (2). However, the staining is patchy within individual glands and sometimes only one or two basal cells are identified (Figure 2). If specific staining occurs in the negative control tissue, patient specimen’s results must be considered invalid.

On needle biopsy, if a small glandular focus is atypical, yet has features suggestive of adenosis, despite being entirely negative for basal cells, an appropriate diagnosis is “Atypical glandular proliferation. Adenosis cannot be excluded”. Partial atrophy and high grade prostatic intraepithelial neoplasia (HGPIN) show similar staining to adenosis. There is often focal and patchy basal cells staining with occasional glands being totally negative for basal cells (Figures 3-4) (7).

CK HMW, CK 5/6 and p63

A pitfall in the use of immunohistochemistry for the diagnosis of prostate adenocarcinoma is false positive staining for basal cell markers. This can occur in several patterns. A type of false positive staining with basal cell markers are uncommon cases of acinar adenocarcinoma.
Figure 2: A) Low magnification of crowded glands of adenosis mimicking carcinoma. B) Higher magnification showing small glands with pale cytoplasm and benign cytology. C) CK HMW stain showing patchy basal cell staining of scattered adenosis glands. Although some glands are negative, these glands are identical morphologically to glands with basal cells and the entire lesion should be considered benign.

Figure 3: A) Partial atrophy. B) Patchy basal cell staining with CK HMW analogous to the staining seen in adenosis.

Figure 4: A) High grade prostatic intraepithelial neoplasia (HGPIN). B) Patchy basal cell staining with p63 in HGPIN glands.
that label focally with CK HMW and less so with p63 in a non-basal cell distribution (Figure 5). This phenomenon can be seen in all grades of prostate cancer, although more commonly encountered in Gleason scores 8-10 (9). Retention of basal cells in early adenocarcinoma is an extremely rare phenomenon even in highly selected consultation material (Figure 6) (8). Therefore this diagnosis should be made with great caution only when there is unequivocal cancer on the hematoxylin and eosin slide, and preferentially after consulting with an expert pathologist.

Non-specific staining seems to depend on the antigen retrieval method used, with the hot plate method showing more non-specific reaction than the pepsin predigestion and microwave retrieval methods (5,9-11). p63 has greater specificity for basal cells compared with CK HMW, showing less non-specific reactions with cancer cells. A unique problem with p63 is aberrant diffuse expression of p63 in acinar adenocarcinoma (Figure 7) (12).

These cases differ from those showing the non-specific staining of basal cell markers in adenocarcinoma described above in three major aspects:

1) the staining for p63 is strong and diffuse within the malignant glands;

2) the majority of cases with aberrant p63 show distinctive morphology of infiltrative glands, nests and cords with atrophic cytoplasm, hyperchromatic nuclei and visible nucleoli; and

3) other basal cell markers such as CK HMW, and CK 5/6 are totally negative. The other differential diagnosis for a malignant lesion with p63 positivity is basal cell carcinoma. Not in favor of the diagnosis of basal cell carcinoma is the total negativity for other basal cell markers such as CK HMW and CK 5/6 in p63-positive prostate cancers along with its positivity for prostatic secretory cell markers such as prostate-specific antigen (PSA).

Apart from high molecular weight cytokeratins and p63, a range of other markers that label basal cells in the prostate has been suggested (e.g. P-cadherin, podoplanin (D2-40), CD109 or BCL2) (13-16). Since the experience with these experimental markers is limited, these are not recommended in a routine setting.

Positive Markers of Malignancy

AMACR (Alpha-methylacyl-CoA racemase)

It has long been a desire of surgical pathologists to complement basal cell markers, which stain negative in carcinoma, with an affirmative positive marker of malignancy. AMACR was the first such candidate positive marker. AMACR is a mitochondrial and peroxisomal enzyme that is involved in beta-oxidation of branched-chain fatty acids and in bile acid biosynthesis (17). It is expressed in various normal tissues, e.g. hepatocytes, renal tubular epithelial cells and gall bladder mucosa, but also in a variety

![Figure 5. A) Adenocarcinoma of the prostate (arrows). B) CK HMW labeling several cancer cells. The positivity is not in a basal cell distribution as seen in adjacent benign glands (right side). C) Same cancer glands are negative for p63.](image-url)
of dysplastic tissues or malignant tumors including colon cancer and papillary renal cancer (18-20). The highest rates of AMACR overexpression (>95% of cases) have been reported for prostate cancer, which has led to its widespread use as a positive diagnostic biomarker; so far, it is the only one that has gained clinical acceptance. In combination with basal cell markers, AMACR staining can significantly increase the diagnostic accuracy and thus help avoiding unnecessary re-biopsies (21-27). Without AMACR, only “atypical glands” would have been reported in some instances.

However, the interpretation of AMACR staining requires experience, since it also introduces new pitfalls. Approximately 20% of small foci of adenocarcinoma on needle biopsy are negative for AMACR. Foamy gland, atrophic, pseudohyperplastic, and hormone-treated carcinomas express AMACR to an even lesser extent (28-29). AMACR expression also lacks specificity. It is as frequently overexpressed in HGPIN as in adenocarcinoma, and certain benign mimickers of adenocarcinoma such as adenosis, partial atrophy and post-atrophic hyperplasia may express AMACR (30). Consequently, it is essential to interpret AMACR in the context of the entire lesion, using it to confirm a morphological impression of malignancy in a focus of suspicious glands. A suspicious glandular focus that fulfills the histological criteria of carcinoma and that is negative for basal cell markers can still be diagnosed as adenocarcinoma even in the absence of AMACR reactivity.

Figure 6. A) Typical case of adenocarcinoma of the prostate with HGPIN gland. B) Carcinoma glands are positive for CK HMW and p63.

Figure 7. A) Adenocarcinoma on both sides of benign glands (arrow) with atrophic appearance and multilayered nuclei. B) p63/CK HMW cocktail with carcinoma positive for p63 only labeling nuclei with surrounding benign glands having positivity in both nuclei (p63) and cytoplasm (CK HMW). C) CK HMW stain with p63 positive carcinoma negative for CK HMW.
A common dual stain includes p63 and AMACR antibodies, but a potential problem with this stain is that p63 may show background staining in the cytoplasm of benign glands, which may be confused with AMACR immunoreactivity. Another problem is that it is more difficult to identify sparse brown p63-positive basal cells in the setting of intense brown AMACR-positive cytoplasm. A triple stain with AMACR labeled with a red chromogen and both p63 and CK HMW labeled with a brown chromogen circumvents this problem (Figure 8).

In a classic case of partial atrophy or HGPIN, these diagnoses can be established even if basal cells are absent and AMACR is positive (Figures 9-10). In other cases, where the glands are suspicious for partial atrophy or HGPIN yet not definitive and the basal stains are negative (+/- AMACR positivity); these lesions should be reported as: “Atypical glands, suspicious for adenocarcinoma.” (Figure 11). Even entirely benign glands can occasionally lack basal cells and express AMACR (Figure 12).

ERG (Ets-related gene product)
The diagnostic value of ERG IHC is now widely under investigation (31,32). A limitation of ERG as an affirmative positive cancer marker is the large fraction of

Figure 8. A) Gleason score 3+3=6 adenocarcinoma. Note admixed benign glands (*) with paler cytoplasm and luminal infolding. B) Cocktail labeling with brown chromogen labeling both basal cell nuclei (p63) and cytoplasm (CK HMW) in benign glands. Carcinoma lacks basal cells. Red chromogen labels cancer cytoplasm (AMACR) and is negative in the benign glands.

Figure 9. A) Classic partial atrophy. B) Cocktail labeling with brown chromogen basal cells (p63 and CK HMW) and red chromogen labeling AMACR. The diagnosis is still partial atrophy despite the lack of basal cells and positive AMACR.
ERG-negative carcinomas. Earlier studies have reported ERG fusion in 15-72% of cases, depending on cohort design, tumor grade, zonal origin and even patient ethnicity, but the mean prevalence in western countries appears to level around 50% (33-43). However, on limited foci of carcinoma on needle biopsy, the positive rate is more 30-40% (Figure 13). HGPIN is also positive for ERG in a minority of cases. Even though these caveats limit the diagnostic value of ERG to detect primary invasive prostate cancer, combined staining of basal cell markers with ERG may be useful in selected (ERG positive) cases.
FASN (Fatty acid synthase)
FASN overexpression in prostate cancer is well described (Figure 14) (44-49). A main difference with AMACR is the more prevalent expression of FASN in normal tissues and HGPIN, which makes it necessary to compare the staining of atypical glands with adjacent clearly benign glands. However, if this comparison is performed, FASN can be helpful, particularly in AMACR negative cases, which almost always are positive for FASN (50, 51).

GOLPH2 (Golgi phosphoprotein 2)
GOLPH2 (GOLM1) is a 73kDa Golgi phosphoprotein of yet unknown function that has been reported in various profiling studies of prostate cancer (52-54). So far, four groups have independently confirmed the strong overexpression of GOLPH2 in prostate cancer at the protein level, which can be used diagnostically in an experimental setting (55-58).

CYCS, ICK and IKBKB
Other candidate positive markers that have been proposed are somatic cytochrome C (CYCS), intestinal cell kinase (ICK) and inhibitor of nuclear factor-kB kinase subunit (IKBKB) in prostate cancer (59). The very limited experience with these markers requires extensive validation, before they can be recommended.

Primary Adenocarcinoma of the Prostate from Secondary Tumors
PSA (Prostate-specific antigen)
Prostate-specific antigen (PSA, KLK3) is a 33 kDa serine protease that is widely used to confirm the prostatic origin of metastatic carcinoma (61). PSA is however not entirely specific for prostate since it has also been detected in carcinomas of the ovary and the breast, including male breast cancer and other tissues, but it still is probably the most commonly used prostate marker (62-64). The panel of PSA, prostein (P501S), and NKX3.1 minimizes false negative immunoreactivity in a poorly differentiated prostatic adenocarcinoma (Figure 15).
PSMA (Prostate-specific membrane antigen)
PSMA is a folate dehydrolase that is strongly expressed by most prostate carcinomas and their metastases (65). In contrast to PSA, PSMA shows increasing expression levels in high grade tumors and metastases, however it is now acknowledged that it is not prostate specific at all, but is rather widely expressed in various solid tumors including renal cancer, gastrointestinal neoplasms and urothelial carcinomas (66-68).

Prostein (P501S)
Prostein’s prostate-specificity has been independently confirmed and several groups have successfully applied prostein IHC to discriminate a prostatic cancer origin from tumors of the colon and the bladder (69-75). Especially, the separation of high grade prostate cancer from urothelial carcinoma can be successfully achieved with a combination of p63 and prostein (76). The biological functions of prostein, which is androgen regulated and mostly localized to the golgi apparatus of the cell, are unclear. However, it is still regarded to be among the best validated immunohistochemical markers of prostatic origin. In cases where PSA is negative, many will be positive for prostein. An additional advantage is the distinctive granular cytoplasmic staining which distinguishes it from other markers in which a weak positive cytoplasmic blush can be more difficult to interpret.

AR (Androgen receptor)
PSA and PSMA are both targets of androgen signaling and the AR itself is also regulated in prostate cancer (77,78). Again, the diagnostic use of AR staining is greatly hampered by the expression of AR in other human tissues and tumors and it can therefore no longer be recommended (Figure 16) (79).

ERG (Ets-related gene product)
Although ERG expression clearly lacks sensitivity in primary prostatic carcinomas (with 50% negatives), it appears to be quite specific for prostatic origin. More specifically, the genomic translocation has not been found in any other carcinoma, whereas the protein level is slightly less indicative since ERG expression is seen in vascular tumors, thymomas and gynecological neoplasms (80,81). It is also possible, that the sensitivity in prostate cancer metastases exceeds that of primary tumors, since TMPRSS2-ERG rearrangement might be more prevalent in metastases (82).

Four studies of independent groups have analyzed ERG rearrangement as a marker for small cell carcinoma of the prostate, which can be difficult to differentiate from small cell carcinomas of other sites (83-86). All four studies found ERG rearrangements detected by FISH exclusively in prostatic small cell carcinomas (range 45-86%) but not in small cell carcinomas of other sites including bladder and lung. In comparison to other markers previously suggested in this respect, ERG clearly outperforms these, including PSA and also prostein, which was found in only 28% of prostatic small cell carcinoma cases (87). The use of ERG to determine a prostatic origin of small cell carcinomas appears to be the best validated contribution of the ERG rearrangement to prostate diagnostics. However, as small cell carcinomas, regardless of the site of origin, are treated the same it is currently questionable as to the need to specifically diagnose small cell carcinoma of the prostate.

Figure 16. Metastatic prostatic adenocarcinoma to cervical lymph node with positivity for AR.
NKX3.1 (Homeobox protein NKX3.1)
Another androgen regulated and mostly prostate-specifically expressed gene is the homeobox gene NKX3.1, which is found expressed primarily in secretory prostatic epithelia of benign and neoplastic cells, but rarely also in benign testis and invasive lobular carcinomas of the breast (88-90). Some researchers have described a loss of NKX3.1 protein in high grade tumors of the prostate and even a prognostic significance (91, 92). One study compared several prostate marker candidates including NKX3.1 and prostein and found both excellent for the discrimination of prostate from urothelial cancer (Figure 17) (73). More recently, Gurel et al. described NKX3.1 as an excellently sensitive and specific prostate cancer marker, outperforming PSA in this regard (93). This discrepancy to earlier studies is explained by a novel, more sensitive NKX3.1 antibody (sensitivity 98.6%, specificity 99.7%).

AMACR (Alpha-methylacyl-CoA racemase)
Even though AMACR is typically overexpressed in prostate cancer, it is not restricted to it but is also present in up to 92% of colorectal adenocarcinomas, as well as breast, lung, ovarian, renal cell carcinomas (especially the papillary variant), as well as bladder urothelial and adenocarcinomas (94-97). Thus, this marker is not useful in the differential diagnosis of prostate cancer from other malignancies.

Specific Differential Diagnoses
Prostate Cancer (PCa) vs. Urothelial Cancer (UC)
Although the morphology of invasive urothelial carcinoma is typically distinct from glandular adenocarcinoma of the prostate, the morphological discrimination from high grade prostate carcinomas can be challenging, particularly in small biopsies. IHC can be helpful, but beware: PSA is mostly negative in UC, but may be missing in high grade prostate cancer. AR is mostly positive in PCa, but is also seen in UC, its use is therefore discouraged. Accumulation and strong nuclear staining of p53 is more prevalent in invasive UC, but may also be positive in high grade PCa. CK7/20 are commonly used markers for UC, however both cytokeratins may also be expressed in high grade PCa, so they lack discriminatory power.

Prostate Cancer (PCa) vs. Colorectal Cancer (CRC)
The typical immunophenotype of CRC is CK20+/CK7-/CDX2+. Of these markers CDX2 alone is helpful, since it is very rarely positive in PCa, however there are exceptions (98). As stated above, prostein and NKX3.1 are helpful to identify PCa. Nuclear staining of beta-catenin is more common in CRC, however this lacks sensitivity and specificity and is discouraged as a marker for CRC.

Diagnosis of Pretreated Prostate Carcinomas
The effects of organ sparing therapy, i.e. androgen ablation and radiotherapy, on prostatic tissues are well documented (60). Reactive changes in benign tissues and tumor atrophy can markedly obscure the morphology. This introduces a risk to over- or underdiagnose prostate cancer and
particularly Gleason scores can be markedly altered, e.g. by an assignment of Gleason pattern 4 to areas that had been Gleason 3 prior to therapy. Treated prostate carcinomas tend to show some loss of AMACR expression, limiting its value in a post-treatment situation. In severely regressed cases, stainings for pan-cytokeratin and basal cell markers are more helpful to ascertain the presence of residual or recurrent prostate cancer (Figure 18).

Pitfalls in the Use of Prostatic Markers

NKX3.1 and prostein (P501S) are the most specific markers for prostate origin (Figures 15-16). They also have the advantage of nuclear and clumpy granular cytoplasmic staining, respectively, in contrast to PSA where non-specific diffuse cytoplasmic staining can be misinterpreted as true positivity (Figure 19). PSMA can be found in rare cases of pulmonary small cell carcinoma, hepatocellular carcinoma, papillary renal cell carcinoma and most importantly in 17% of urothelial carcinomas (99). The two oldest prostatic markers that exist are PSA and PSAP. PSAP is relatively specific although it suffers from relative decreased sensitivity. Situations that can cause diagnostic difficulty include PSA and PSAP within periurethral glands, as well as cystitis cystica and cystitis glandularis in both men and women (100-102). Other examples of cross-reactive staining include anal glands in men (PSA, PSAP) and urachal remnants (PSA) (103,104). Some intestinal carcinoids and pancreatic islet cell tumors are strongly reactive with antibodies to PSAP, yet are negative with antibodies to PSA (105).

Figure 18. A) Adenocarcinoma of the prostate with radiation effect. B) Cocktail stain with benign prostate glands with radiation effect (right side) labeling basal cells brown with CK HMW and p63. Carcinoma with treatment effect (left side) lacks basal cells and is positive for AMACR (red).

Figure 19. A) Specific clumpy granular staining of P501S in benign prostate gland. B) Weak diffuse nonspecific biotin labeling in a case labeled with P501S that can be correctly diagnosed as being negative. If this was a PSA stain, then it may have been incorrectly called positive.
tigen can be falsely negative. Another pitfall is negative staining with prostate markers in poorly differentiated adenocarcinomas of the prostate, which is the situation where this immunohistochemistry is typically performed. PSA immunexpression is inversely correlated with increasing Gleason score, and a minority of Gleason score 10 adenocarcinomas may be negative for PSA, especially in limited material. P501S and NKX3.1 expression seems to be unrelated to Gleason grade. It is important to note that a small minority (less than 5%) of poorly differentiated prostatic adenocarcinomas are totally negative for all prostatic markers (76). Therefore, the lack of immunoreactivity for prostate-specific markers in a poorly differentiated tumor, especially if present in limited amount (in biopsy specimens), does not totally exclude the diagnosis of a poorly differentiated prostatic adenocarcinoma.

Concluding Remarks
The increasing number of biopsies, time constraints and the demands of quality management and legal issues have prompted pathologists to adapt their workflow accordingly and to increase their diagnostic efficiency. Over the past 20 years, immunohistochemistry has become an indispensable tool in surgical pathology and some areas, like lymphoma classification, even depend strictly on immunophenotyping. In the evolution of current concepts of prostate pathology, immunohistochemistry has also become increasingly important.

In this review, we aimed to illustrate that immunohistochemistry can in fact be immensely contributive in diagnostic prostate pathology, if used with care and experience. No single marker can establish a diagnosis on its own, but has to be used in close conjunction and with a thorough assessment of the individual cases’ morphological as well as the clinical context, to lead to correct conclusions for improved patient care. Every tool has pros and cons. The generally increased diagnostic certainty achieved with immunohistochemistry also opens up the possibility of new pitfalls that the pathologist must be aware of.

We have compiled this review to cover the most important uses and pitfalls of contemporary immunohistochemistry in prostate diagnostics and hope that this may be a helpful companion in daily work.

Dako Antibodies for Prostate Tissue Antigens

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*Not available in the US.
Prostate Pathology | IHC

Stains using Dako FLEX RTU antibodies

**AMACR**
Clone 13H4
Prostate adenocarcinoma. The majority of cells show a distinct granular cytoplasmic staining reaction and the benign glands are mostly negative.

**AMACR + CK HMW + CK 5/6**
Clones 13H4 + 34ßE12 + D5/16 B4
Prostate. Cells labeled by Anti-AMACR antibody display a distinct red cytoplasmic granular staining. Cells labeled by Anti-CK HMW and Anti-CK 5/6 antibody display strong brown cytoplasmic staining.

**Cytokeratin 5/6**
Clone D5/16 B4
Prostate hyperplasia and prostate carcinoma. The normal and benign glands show a distinct cytoplasmic staining reaction in the basal cells.

**Cytokeratin HMW**
Clone 34ßE12
Prostate adenocarcinoma. Various staining reaction patterns are seen: Continuous cytoplasmic staining in normal gland, discontinuous pattern in PIN and no staining in invasive cancer cells.

**ERG**
Clone EP111
Prostate adenocarcinoma. The majority of neoplastic cells show a moderate to strong nuclear staining reaction.

**Ki-67**
Clone MIB-1
Tonsil. The germinal center B cells show a moderate to strong nuclear staining reaction.
p53 protein  
Clone DO-7  
Breast carcinoma, the neoplastic cells show a moderate to strong nuclear staining reaction.

p63 protein  
Clone DAK-p63  
Benign prostate hyperplasia and normal prostate. The normal and benign glands show a distinct nuclear staining reaction in basal cells, while the secretory and neoplastic cells are negative.

Prostein (P501S),  
Clone 10E3  
Prostate adenocarcinoma. The majority of neoplastic cells show a moderate to strong granular cytoplasmic staining reaction.

Prostate-Specific Antigen (PSA)  
Polyclonal  
Prostate adenocarcinoma. The neoplastic cells and the hyperplastic glands show a moderate to strong and diffuse cytoplasmic staining reaction.

Prostate-Specific Membrane Antigen (PSMA)  
Clone 3E6  
Prostate adenocarcinoma. The majority of neoplastic cells show a moderate to strong cytoplasmic and/or membranous staining reaction.
References


Relentless in our commitment to fighting cancer. Together.