Zyto-Facts 1-2014

News for Pathology and Immunohistochemistry

Speaking to You

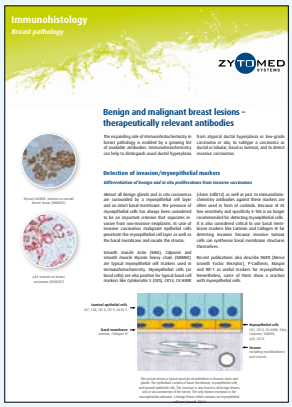
Karl-Georg Lintermann PhD,
Export manager

I would like to welcome all readers of this issue of our Newsletter Zyto_Facts 1-2014. My name is Karl-Georg Lintermann. After 11 years of experience in sales of histochemistry products, I recently took over the responsibility for Zytomed Systems export management.
My aim is to provide you with outstanding reagents for diagnostics and research.
In addition to articles addressing new markers for cancer diagnostics like p40 and SOX-10, this new issue of Zyto_Facts includes information about the stability of our reagents in the "Focus: lab work" section.
Last but not least, I would like to point out that we took over the production of cell control arrays from our former supplier which allows us to produce these control blocks in accordance to our own high quality standards. The first result of this development is the relaunch of our popular Virus Cell Control Array.

Enjoy reading!
Karl-Georg Lintermann

+++ Newsflash +++ Newsflash +++

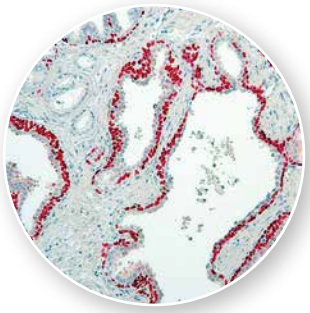
- **Breast cancer: Benign and malignant breast lesion-therapeutical relevant antibodies**
  Please, download the 4 pages brochure directly from our homepage
  www.zytomed-systems.com



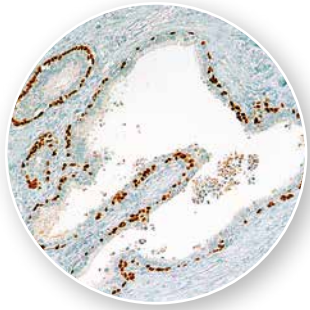
- **38th European Congress of Cytology 2014**
  27–30 Sep 2014, Centre International de conférence Genève (CICG), Geneva, Switzerland
- **30th Congress of the International Academy of Pathology (IAP)**
  5–10 Oct 2014, Bangkok Convention Centre at Centralworld, Bangkok, Thailand
- **Herbsttagung 2014 der ÖGPath-IAP Austria**
  16–18 Oct 2014, Congress Graz, Graz, Austria
- **NordiQC: Diagnostic Immunohistochemistry for Pathologists**
  16–17 Oct 2014, Jagiellonian University, Krakow, Poland,
- **Carrefour Pathologie**
  17–21 Nov 2014, CNIT, Paris La Défense, France
- **USCAP 2015**
  21-27 March 2015, Hynes Convention Center Boston, MA, USA

p40 or p63 – Which Is the Best Marker for Prostate Basal Cells?

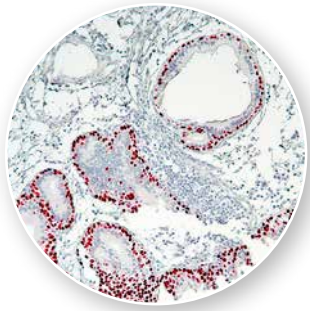
p63 is one of the standard markers for basal cells of the prostate gland and is widely used to distinguish between adenocarcinoma and squamous cell carcinoma of the lung.
The anti p40 antibody directed against an N-terminal truncated form of the p63 protein (ΔNp63) is currently replacing p63 as several studies [1, 2] showed higher specificity of p40 for squamous cells than p63.
Until recently no studies addressed p40 staining in prostate cancer were available.
Sailer *et al.* from the Charité in Berlin, addressed the question whether p40 or p63 is the marker of choice in prostate cancer diagnostics [3].
Immunostaining was performed on 640 malignant and normal prostate tissues. The most widely used p63 clone 4A4 was compared to the p40 polyclonal antibody in a semiquantitative manner. p40 like p63 is detected in prostate basal cells in normal and premalignant prostate glands. Staining pattern of normal tissue was almost identical. More important differences were seen in staining of carcinomas: 1.4% of the nuclei were positive with the antibody p63 whereas only 0.6% stained positive for p40.
Therefore the authors concluded that p40 is as reliable in prostate diagnostics as p63. They emphasised the higher specificity of p40 as it showed less than half of false positive staining of aberrant cells.
The polyclonal p40 antibody used in this study showed cytoplasmic background in some cases. The recently developed monoclonal p40 (BC28) shows no background and is especially useful in cocktails with P504S, a marker for prostate cancer and premalignant stages.
A new study comparing the performance of the new monoclonal p40 antibody with that of the polyclonal in lung carcinoma demonstrates that both sensitivity and specificity are the same [4]. The p40 monoclonal staining was observed in a greater percentage of urothelial carcinomas, squamous and basal cell skin cancers, as well as in head and necks cancers of squamous cell origin when compared to p63. In prostate no adenocarcinoma showed positiv staining for p40 with the monoclonal antibody.
The p40 expression in basal cells of prostate glands and prostatic intraepithelial neoplasia were almost identical to those of p63. It seems that p40 is the superior marker both in lung and prostate diagnostics.
In summary it seems that p40 is the best marker both for lung and prostate diagnostics.



p40 ready-to-use (clone BC28) staining of benign glands in prostate carcinoma (BMS050)



p40 ready-to-use (clone BC28) staining of benign glands in prostate carcinoma (BMS050)



p40 (polyclonal) staining of benign glands in prostate carcinoma (RBK054)

Bibliography

[1] Nonaka D. A study of DeltaNp63 expression in lung non-small cell carcinomas. Am J Surg Pathol 36:895–899, 2012

[2] Bishop JA *et al.* p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Modern Pathology 25:405–415, 2012

[3] Sailer V *et al.* Comparison of p40 (ΔNp63) and p63 expression in prostate tissues-which one is the superior diagnostic marker for basal cells? Histopathology 63:50-56, 2013

[4] Tacha D *et al.* An immunohistochemical analysis of a newly developed, mouse monoclonal p40 (BC28) in lung, bladder, skin, breast, prostate, and head and neck cancers. Arch Pathol Lab Med. 2014 Feb 14. [Epub ahead of print]

▶ Product information

Description	Reactivity	Method	Pretreatment	Dilution	CE/IVD	Volume	Cat. No
p40 Clone: BC28 Host: Mouse	HU	P | F	HIER in Citrate pH6.0	Ready-to-use	✓	16 ml	BMS050
				1:50 – 1:200		0.5 ml	MSK097-05
						1 ml	MSK097
p40 Clone: polyclonal Host: Mouse	HU	P	HIER in Citrate pH6.0 or EDTA pH 9.0	Ready-to-use	✓	6 ml	RBG054
				1:50 – 1:200		0.5 ml	RBK054-05
P504S (AMACR) Clone: polyclonal Host: Rabbit	HU	P	HIER in EDTA pH 9.0	Ready-to-use		16 ml	BRB035
				1:50 – 1:100		0.5 ml	RBK002-05
						1 ml	RBK002

SOX-10 – An Underutilized Marker in Melanoma Diagnostics?

SOX-10 is a member of the sex-determining region Y-related HMG-box family. This neural crest transcription factor is crucial for specification, maturation, and maintenance of Schwann cells and melanocytes. The study of Nonaka *et al.* describes SOX-10 as a sensitive marker for malignant melanoma [1]. SOX-10 nuclear expression was found in 97 % of melanomas whereas S-100 protein was expressed in 91 % of melanomas. In contrast to other markers of melanocytes, SOX-10 detects spindle cell and desmoplastic melanoma with high sensitivity (100 %).

This fact is especially important when the tumour mimics other spindle cell lesions with S-100 positivity and does not express melanoma-specific markers, such as HMB-45, Melan A, Tyrosinase, and MiTF. Using SOX-10 immunohistochemistry metastatic melanoma in sentinel lymph nodes can be distinguished from S-100-positive interdigitating dendritic cells, follicular dendritic cells and Langerhans cells in the lymph node.
Fibroblasts and histiocytes that could histopathologically mimic melanoma cells show weaker SOX-10 staining compared to S-100 and MiTF making the interpretation of immunostainings easier [2].
SOX-10 immunohistochemistry can be used to detect peripheral nerve sheath tumour with schwannian differentiation, such as neurofibroma (96–100 % positivity) and schwannoma (100 %). In addition SOX-10 shows a higher sensitivity and specificity than S-100 for malignant peripheral nerve sheath tumours [1].

A recent review of Nelson Ordóñez describes SOX-10 as a useful immunohistological marker for a variety of diagnostic applications [3] and Cary Buresh from ProPath summarizes his experiences with SOX-10 as follows:

"... Sox10 is a superb nuclear marker of melanocytic lesions.... And it is also an excellent marker of Schwann cells and myoepithelial cells in a variety of tumours... We have been using Sox10 with great success ... and we think that this antibody is underutilized."

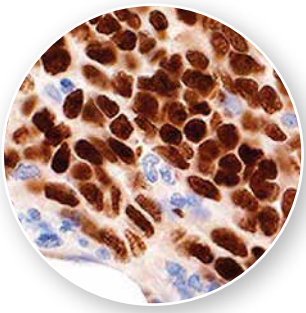
Bibliography

[1] Nonaka D *et al.* Sox10: A pan-schwannian and melanocytic marker. Am J Surg Pathol 32:1291-1298, 2008

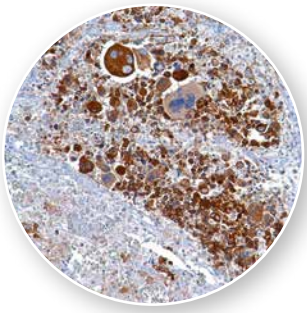
[2] Ramos-Herberth FI *et al.* SOX10 immunostaining distinguishes desmoplastic melanoma from excision scar. J Cutan Pathol 37:944-952, 2010

[3] Ordóñez NG. Value of SOX10 immunostaining in tumor diagnosis. Adv Anat Pathol 20:275-283, 2013

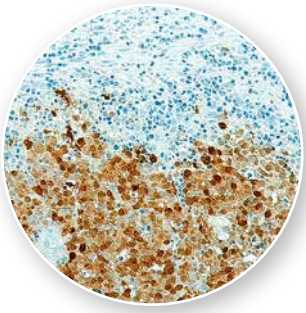
[4] Buresh CJ. Propath-Newsletter: Immunohistochemistry. July 2010 (propath.com)



SOX-10 (RBK057-05) staining on melanoma (DAB)



Melan A (MSK056) staining on melanoma (DAB)



S-100 (MSK050) staining on melanoma (DAB)

▶ Product information

Description	Reactivity	Method	Pretreatment	Dilution	Volume	Cat. No
SOX-10 Clone: polyclonal Host: Rabbit	HU	P	HIER in EDTA pH9.0	Ready-to-use	6 ml	RBG057
				1:25 – 1:100	0.5 ml	RBK057-05

Focus: lab work Stability of Zytomed Systems Reagents

At Zytomed Systems, a strict quality management system (ISO9001/ISO13485) is implemented to provide you with products complying with the highest level of sensitivity and specificity until the expiry date - and beyond.

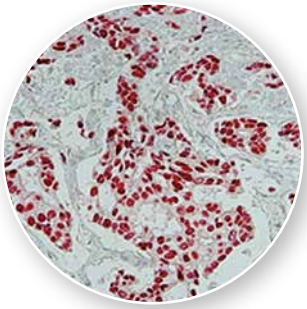
Our reagents, like antibodies, buffers, detection systems, and chromogens are permanently tested for stability. Shelf life is monitored by real-time and real-temperature testing during a pre-determined period of time. In addition we perform high-temperature accelerated stability tests at 37° C.

Stability of antibodies used for immunohistochemistry is a major concern in many labs. Antibody storage ‚shelf life' may range from weeks to many years and depends on the specific characteristics of the antibody and storage conditions. A number of diagnostic antibodies have been shown to preserve their functionalities after years of storage at 4° C.

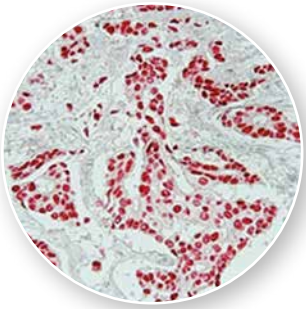
We recommend to aliquot concentrated antibodies in order to avoid multiple pipetting from a single vial. This can help minimizing contamination with spoiling microorganisms or proteases. If possible, use sterile pipette tips when preparing the aliquots. Do not prepare antibody aliquots < 20 µl. The smaller the aliquot, the more the stock concentration is affected by evaporation and adsorption of the antibody onto the surface of the storage vial. If specified in the datasheet, it might be necessary to freeze aliquots for long term storage. Avoid damage due to freezing and thawing and keep a thawed antibody aliquot at 4° C.

Most antibodies diluted from concentrates in high quality dilution buffer (like our Antibody Diluent ZUC025) are stable for more than 24 months. As for aliquoting we recommend sterile pipette tips for preparing dilutions from stock solutions. We suggest using diluted antibodies for up to 4 weeks in daily routine work. It is not necessary to prepare ready-to-use antibodies from concentrates weekly or even right before use. Reducing the number of antibody dilutions from the concentrate helps to minimize dilution mistakes, to avoid contamination of the stock and, last but not least, to increase reproducibility of your IHC stainings.

Our own in-house testing demonstrates the outstanding stability of our reagents (which is reflected by the daily experience of our customers). The first two pictures demonstrate the stability of primary antibodies after dilution.

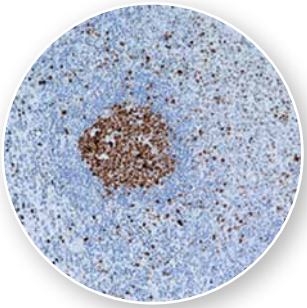


ER (BMS008) staining on breast cancer 2 months after dilution

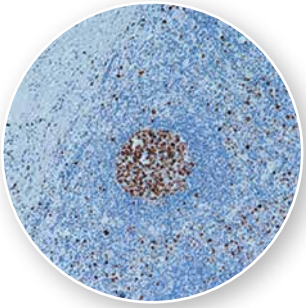


ER (BMS008) staining on breast cancer 24 months after dilution

The following two pictures show the detection of Ki-67 using the One-Step Polymer ZUC053. Only minor differences in staining intensity can be observed even though one of the detection systems was expired 14 month before use.

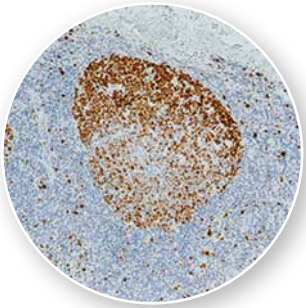


Ki-67 (RBK027) immunostain, ZytoChem Plus (HRP) One-Step Polymer (ZUC053) new batch

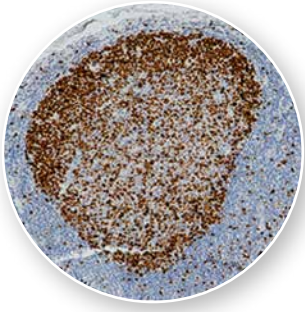


Ki-67 (RBK027) immunostain, ZytoChem Plus (HRP) One-step Polymer (ZUC053) expired 14 months ago

The next two pictures illustrate Ki-67 (RBK027) immunohistochemistry using a Two-Step Polymer Detection System (POLHRP100). Expired Antibody Diluent (ZUC025) and expired HIER Citrate Buffer pH 6.0 (ZUC028) for pre-treatment was used respectively.



Antibody Diluent (ZUC025) expired 16 months ago



HIER Citrate Buffer (ZUC028) expired 15 months ago

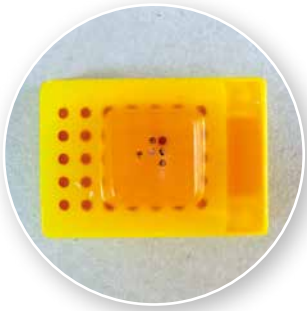
Although we do not recommend using antibodies and reagents for immunohistochemistry after their expiry date Zytomed Systems put a lot of efforts to ensure constant good quality over the complete shelf life of our products.

Cell Control Array Virus

We are happy to relaunch the Cell Control Array Virus. It is designed for the qualitative control of immunochemical staining and *in situ*-hybridisation of virus infected tissue. The paraffin block contains cores of CMV, HSV type 1 and type 2, EBV and Polyomavirus/SV40 infected cell lines. In addition a core of heart muscle is included in the block.

The small size of the control block sections allows for simultaneous mounting of patient tissue and a section from the control block on the same slide. Thus, you will have an on-slide control array staining (OSCAR) proving a regular stain even after years of storage.

Recently we took over the production of our Cell Control Arrays from our former supplier company multiblock GmbH. Production is now done in-house at Zytomed Systems and is implemented in our quality management system (ISO 9001:2008 and ISO13485:2003). Via this step we are able to ensure a constant excellent quality and permanent availability of all Cell Control Arrays for you.



Cell Control Array Virus
(MB-CC VIR)

EBV *in situ*-hybridization of Cell Control Array Virus
(MB-CC VIR)

CMV Imunostaining of Cell Control Array Virus
(MB-CC VIR)

Cell Controll Arrays		
MB-CC REZ	Cell Control Array Receptor	Control Cell Block including cell lines expressing certain levels of ER, HER2, PR
MB-CC HPV	Cell Control Array HPV	Control Cell Block including cell lines expressing proteins of different HPV types
MB-CC BAC	Cell Conrol Array Bacteria plus Fungi	Control Cell Block including different bacteria types and fungi
MB-CC VIR	Cell Control Array Virus	Control Cell Block including cell lines expressing proteins of different viruses

MAX-Line Ready-to-Use Antibodies – New Specificities Available:

In the daily routine of the immunohistochemistry lab ready-to-use antibodies are a convenient alternative to concentrated formats. Using prediluted antibodies helps to avoid mistakes in diluting from concentrates. In addition, ready-to-use antibodies provide you with standardised antibody concentrations. Our high volume Max-Line antibodies in 16 ml-format combine these advantages with low costs per test. Moreover, most of them are CE/IVD certified.

- CK5/14 (BMS023) – a superior marker for squamous cell epithelia.
- The rabbit monoclonal Estrogene receptor, SP1 (BRB053) is now available as CE/IVD.

Product information

Description	Clone	Cat. No.
Actin (smooth muscle)	1A4	BMS001
Bcl-2	Bcl-2-100	BMS021
Calretinin	polyclonal	BRB022
CD3	SP7	BRB041
CD4	SP35	BRB042
CD8	SP16	BRB036
CD10 (CALLA)	56C6	BMS043
CD20	L26	BMS003
CD31	JC70	BMS044
CD34	QBEnd/10	BMS045
CD45 (LCA)	PD7/26 & 2B11	BMS046
CD56	RCD56	BRB039
CD79a	JCB117	BMS005
CDX-2	EPR2764Y	BRB028
CEA	Col-1	BMS029

Description	Clone	Cat. No.
Chromogranin A	LK2H10	BMS018
Cytokeratin 5/6	D5/16B4	BMS017
Cytokeratin 5/14	XM26 & LL002	BMS023
Cytokeratin 7	OV-TL 12/30	BMS030
Cytokeratin 20	Ks20.8	BMS037
Cytokeratin HMW	34ßE12	BMS015
Cytokeratin pan	AE1 & AE3	BMS006
Desmin	D33	BMS007
E-Cadherin (CD324)	EP700Y	BMS021
Epithelial Specific Antigen (Ep-CAM)	Ber-EP4	BMS048
ER	1D5	BMS008
ER	SP1	BRB053
GLUT1	polyclonal	BRB049
Helicobacter pylori	polyclonal	BRB032
HER2/neu	CB11	BMS014

Description	Clone	Cat. No.
Ki-67	SP6	BRB040
Melanoma	HMB45	BMS010
MLH1	G168-15	BMS033
MSH2	FE11	BMS034
p40	BC28	BMS050
p63 *	4A4	BMS052
P504S *	polyclonal	BRB035
Pan Basal-Cocktail (p63 & CK5/14) *	4A4 & XM26 & LL002	BMS051
Pan B-Cocktail (CD20 & Pax-5) *	L26 & polyclonal	BMS026
Pax-5	polyclonal	BRB027
PR	SP42	BRB038
S-100	4C4.9	BMS013
TTF-1	8G7G3/1	BMS016
Vimentin	V9	BMS012

* Antibody for research use only. All other antibodies are CE/IVD certified.

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