

Zyto-Facts¹⁻²⁰¹⁵

News for Pathology and Immunohistochemistry



ZYTOMED
SYSTEMS

Editorial



Karl-Georg Lintermann PhD,
Export manager

The holiday season is turning to its end and Zytomed Systems starts the autumn season with new interesting antibodies for you.

We recently launched Cytokeratin Pan Plus, an antibody cocktail which detects more cytokeratins than other pan cytokeratin antibody mixes. The differential diagnostic of lobular versus ductal carcinoma in situ is made easier and more reliable by our new rabbit monoclonal E-Cadherin antibody. Both antibodies are a valuable addition to your marker panel for differential diagnostics. Finding the primary site of a metastatic tumour can be a challenging task. Immunohistochemistry is the gold standard for this diagnosis; but not all markers keep their sensitivity in undifferentiated neoplasias and metastasis. Find out more on page three.

Positive (and negative) control tissue in immunohistochemistry is increasingly used to ensure consistent quality of stains. These pages hold the answer to the frequently asked question: How to store my positive control slides?

Enjoy reading!
Karl-Georg Lintermann

++ Newsflash +++ Newsflash ++

► **Herbsttagung 2015 der ÖGPath- IAP Austria**
8-10 Oct 2015,
Klinikum Wels-Grieskirchen,
Wels, Austria

► **NordiQC Course in Diagnostic Immunohistochemistry for Pathologists**
12-13 Oct 2015,
Jagiellonian University,
Krakow, Poland

► **ASCP 2015 – American Society for Clinical Pathology**
28-31 Oct 2015,
Long Beach Convention Center,
Long Beach CA, USA

► **Carrefour Pathology**
3-6 Nov 2015,
Le Palais des Congrès de la Porte Maillot,
Paris, France

► **MEDICA 2015**
16-19 Nov 2015,
Messe Düsseldorf, Düsseldorf, Germany

► **XXVIII World Congress World Association of Societies of Pathology and Laboratory Medicine**
18-21 Nov 2015,
Cancun Center, Cancun, Mexico

► **USCAP 2016**
12-18 Mar 2016,
WSCC – Washington State Convention Center,
Seattle WA, USA

E-Cadherin and ductal breast carcinomas

A new rabbit monoclonal antibody enhances specificity and sensitivity

E-Cadherin is a member of a protein superfamily which is characterized by calcium dependent membrane proteins. E-Cadherin belongs to the classic Cadherin subfamily and like all members of this family is involved in cell adhesion. Loss of adhesiveness is thought to be an important step in the development of local invasion. Consequently it has been shown that loss of expression of E-Cadherin is closely related to the progression of various carcinomas and poor prognosis. In prostate cancers, for example, the expression of E-cadherin is reduced or absent in comparison with its strong expression in normal prostate. In breast carcinoma a correlation was observed between low expression of E-cadherin, lymph node metastases and poor prognosis. Overall, reduced E-Cadherin expression

is seen in about 45% of tumours in a variety of organs.

The most important use of E-Cadherin antibodies is the differentiation of ductal vs lobular carcinoma of the breast (DCIS vs LCSC). Strong E-Cadherin immunostaining is detected in ductal carcinoma, whereas lobular carcinomas display a complete loss of E-cadherin expression.

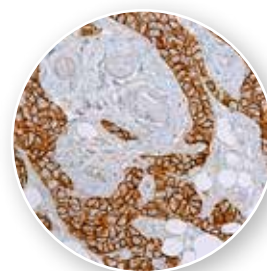
Catenin delta (p120) is associated to E-Cadherin and produces a complex linked to the actin filament network which seems to be of primary importance for cell-adhesion properties of cadherins

Therefore E-Cadherin is located in the membrane of DCIS and loss of E-Cadherin expression in LCIS leads to cytoplasmic staining of the Catenin delta antibody [1].

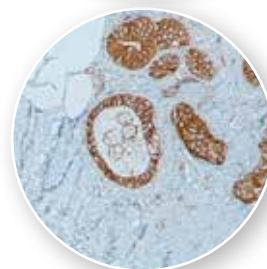
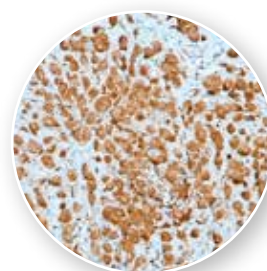
	DCIS	LCIS
E-Cadherin	+	-
Catenin delta (p120)	membrane	cytoplasm

A new study compared immunoreactivity of the new rabbit monoclonal EP700Y to the mouse HECD1 clone in breast carcinoma. Both clones showed excellent specificity (100% in this study) but EP700Y show-

ed an increased sensitivity (99% vs 93%) in ductal carcinoma. In addition using clone ER700Y increased signal intensity substantially, which facilitates interpretation of stained breast carcinomas [2].



E-Cadherin clone EP700Y staining on breast carcinoma



Staining of p120 Catenin delta on LCIS: cytoplasmic localisation in carcinoma cells, membranous staining in adjacent ducts

Product information

Description	Reactivity	CE/IVD	Pre-treatment	Dilution	Volume	Cat. No.
E-Cadherin Clone: EP700Y Host: Rabbit	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	RBG043
				1:50-200	16 ml	BRB047
					0.5 ml	RBK043-05
					1 ml	RBK043
p120, Catenin (delta) Clone: SP63 Host: Rabbit	Human	-	HIER in EDTA pH 9.0	Ready-to-use	7 ml	503-3631
				1:100	0.5 ml	503-3632
					1 ml	503-3634

Bibliography

- [1] Dabbs DJ *et al.* Lobular versus ductal breast neoplasms: the diagnostic utility of p120 catenin. *Am J Surg Path* 31:427-437, 2007
- [2] Hoang LL *et al.* A new rabbit monoclonal E-Cadherin antibody (EP700Y) shows higher specificity than mouse monoclonal E-Cadherin (HECD-1) antibody in breast ductal carcinomas and does not stain breast lobular carcinomas. *App Immunohistochem Mol Morphol* 22:606-612, 2014

CUP (Cancer of unknown primary)-Syndrome

Identifying the primary by lineage specific transcription factors

CUP-Syndrome is defined by metastatic disease in which the primary site could not be identified (based on clinical history, complete physical examination, routine laboratory tests, imaging and radio-metabolic techniques). In these cases, metastatic cells do not display morphology and properties referable to the tissue/organ in which they are arising and can not be related to any suspected primary site. These tumours represent 3-5% of all cancers.

Although the overall prognosis of CUP patients is poor, it is possible to distinguish subgroups of patients with favourable prognosis by identifying a predicted primary site. This will be increasingly important as advanced therapies i.e. targeted therapies will emerge.

At the present immunohistochemistry (IHC) is the gold standard for identifying putative primaries. Several panels of specific antibodies have been proposed containing antibodies against cytokeratins and lineage specific cytoplasmic and nuclear antibodies.

An often underestimated fact is the poor sensitivity of cytoplasmic markers like Mammaglobin, GCDFP-15, HePar-1, and others, in poorly differentiated tumours. Unlike cytoplasmic proteins, transcription factors are often involved in lineage commitment and organ specific development and thus their expression is more conserved even in poorly differentiated tumours and metastases.

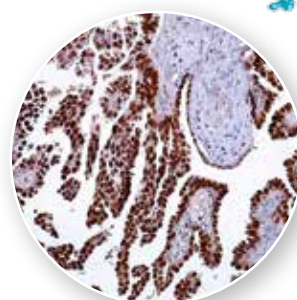
A recently published review addresses this issue and describes useful nuclear marker in detail [1].

CDX-2 is a caudal related homeobox transcription factor implicated in intestinal development showing a very high sensitivity in gastrointestinal primaries and metastases [2]. Although there is some loss in sensitivity in high grade tumours and advanced gastric carcinomas CDX-2 is the most sensitive and commonly used marker for gastrointestinal origin.

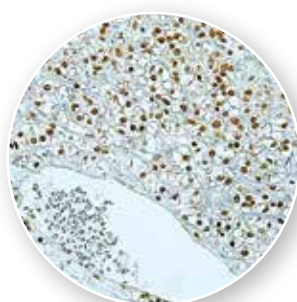
Pax-8 is a member of the paired box transcription factor family. It has become a frequently used marker due to the excellent sensitivity and specificity for carcinomas originating from the ovary, kidney and thyroid, whereas carcinomas from lung, bladder, and breast are mainly negative for PAX-8. Comprehensive studies showed the superiority of PAX-8 in the context of CUP [3-5].

Thyroid transcription factor 1 (TTF-1) is a long established marker for thyroid and lung. It is mainly used to ascribe lung origin to metastatic and primary adenocarcinomas as lung is often a site of metastasis and lung carcinomas are commonly encountered in other sites presenting as CUP.

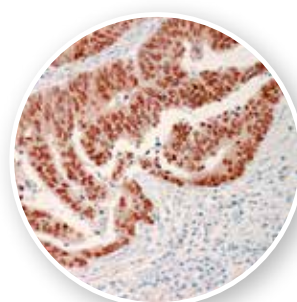
In addition TTF-1 clone 8G7G3/1 stains the cytoplasm of benign and neoplastic hepatocytes due to cross-reactivity, most probably to the antigen of HePar-1. Together with other immunohistochemical support this can be used to distinguish HCC from histologic mimics [6].



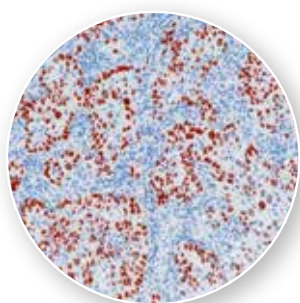
Pax-8 staining
on ovarian carcinoma



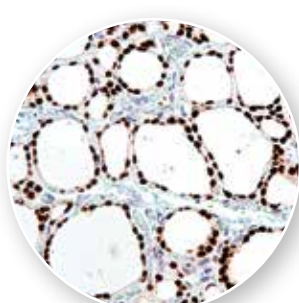
PAX-8 staining
on clear cell RCC



CDX-2, liver metastasis
of colon carcinoma



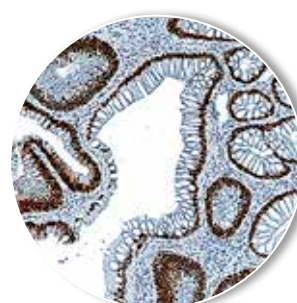
TTF-1 staining on
pulmonary adenocarcinoma



TTF-1 staining
on thyroid gland



Cytoplasmatic staining of TTF-1
(8G7G3/1) on hepatocytes



CDX-2 staining
on colon

► Product information

Description	Reactivity	CE/IVD	Pre-treatment	Dilution	Volume	Cat. No
CDX-2 Clone: EPR2764Y Host: Rabbit	Human	✓	HIER in Citrate pH6.0	Ready-to-use	16 ml	BRB028
				1:50 – 1:100	1 ml	RBK019
					0.5 ml	RBK019-05
PAX-8 Clone: Polyclonal Host: Rabbit	Human	✓	HIER in Citrate pH6.0	Ready-to-use	6 ml	RBG047
				1:25 – 1:50	0.5 ml	RBK047-05
TTF-1 (Thyroid Transcription Factor 1) Clone: 8G7G3/1 Host: Mouse	Human, Rat	✓	HIER in Citrate pH6.0	Ready-to-use	16 ml	BMS016
				1:200 – 1:500	1 ml	MSK004
					0.5 ml	MSK004-05

Bibliography

- [1] Conner JR, Hornick, JL. Metastatic carcinoma of unknown primary: Diagnostic approach using immunohistochemistry. *Adv Anat Pathol* 22:149-167, 2015
- [2] Werling RW *et al.* CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol* 27:303-310, 2003
- [3] Laury AR, *et al.* A comprehensive analysis of PAX8 expression in human epithelial tumors. *Am J Surg Pathol* 35:816-826, 2011
- [4] Ozcan A, *et al.* Pax8 expression in non-neoplastic tissues, primary tumors, and metastatic tumors: a comprehensive immunohistochemical study. *Mod Pathol* 24:751-764, 2011
- [5] Woodard AH *et al.* NY-BR-1 and PAX8 immunoreactivity in breast, gynaecologic tract, and other CK7+ carcinomas: potential use for determining site of origin. *Am J Clin Pathol* 136:428-435, 2011
- [6] Gu K, *et al.* Cytoplasmic immunoreactivity of thyroid transcription factor-1 (clone 8G7G3/1) in hepatocytes: true positivity or cross reaction? *Am J Clin Pathol* 128:382-388, 2007

ZYTOMED SYSTEMS

Efficient immunohistochemical differential diagnosis of undifferentiated neoplasia

Conventional histomorphology: plasmoblastic and/or high-grade spindle-cell

Neoplasm	Marker	Result
Plasmoblastic	CD138	+
	CD139	+
	CD146	+
	CD147	+
High-grade spindle-cell	CD138	+
	CD139	+
	CD146	+
	CD147	+

Conventional histomorphology: epithelial, intermediate-cell to large-cell

Neoplasm	Marker	Result
Epithelial	CD138	+
	CD139	+
	CD146	+
	CD147	+
Intermediate-cell to large-cell	CD138	+
	CD139	+
	CD146	+
	CD147	+

Conventional histomorphology: small-cell

Neoplasm	Marker	Result
Small-cell	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
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Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
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GEP	CD138	+
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GEP	CD138	+
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GEP	CD138	+
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GEP	CD138	+
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Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
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	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
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	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

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	CD139	+
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	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

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GEP	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
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	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
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	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Excludes all Different Patterns)

Neoplasm	Marker	Result
GEP	CD138	+
	CD139	+
	CD146	+
	CD147	+

Markers for immunohistochemical diagnosis of GEP (Ex

Focus: lab work

How to store positive control slides

Best preservation of antigens by correct storage

Quality control and validation has become an increasingly important issue in immunohistochemistry. Using external positive and negative control tissue is the key to achieve high quality and reproducibility of your immunostains.

To cut control tissue in advance is recommended, whether on-slide-controls or control tissues on a separate slide are used.

A frequently asked question is how to store control slides properly.

To address this issue Zytomed-Systems laboratory conducted a study which shows the influence of storage conditions on immunostains.

Tissue was cut and stored for 5 month under different conditions. To emphasize the influence of different parameter even extreme storage conditions were used. Immunohistochemistry was done in parallel with freshly cut tissue of the same paraffin block and the staining was compared by microscopy.

Lobular breast cancer was used as control tissue. 95% of tumour cells were Estrogen receptor (ER) positive and proliferation rate was 15%.

These reagents were used in the study:

ER (clone 1D5), dilution factor 1:200 (Cat. No.

MSK001) and **Ki-67** (clone K-2) dilution factor 1:300 (Cat. No. MSK018). Detection system: **ZytoChem Plus (AP) Polymer Bulk Kit** (Cat. No. POLAP-100), Chromogen: **Permanent AP-Red Kit** (Cat. No. ZUC001-125).

Both antibodies were incubated on formalin-fixed paraffin-embedded tissue after the following storage conditions:

1. Reference tissue: Freshly cut tissue, dried at 37°C overnight.
2. Paraffin section stored for 5 month in a sealed box at 4°C in a fridge.
3. Paraffin section stored for 5 month in a sealed box at room temperature (RT, 22°C).
4. Paraffin section stored for 5 month in an open box at 37°C (incubator).
5. Paraffin section stored for 5 month in a closed humid chamber at room temperature (22°C).

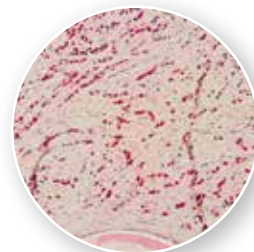
Strongest staining was obtained using freshly cut paraffin sections. Good results were observed after dry storage at 4°C or room temperature. The slides stored at 37°C showed significantly lower staining results. Weak or even negative staining was observed after storage in a humid chamber at room temperature. In addition morphology of the tissue was very poor.

Taken together:

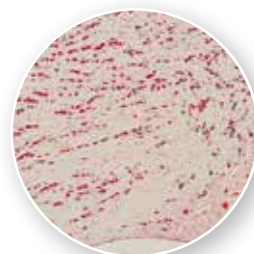
- ▶ Influence of storage temperature:
Freshly prepared > 5 month at 4°C > 5 month at RT >> 5 month at 37°C
- ▶ Influence of humidity:
Freshly prepared > 5 month dry at RT >>> 5 month humid at RT

Our conclusion:

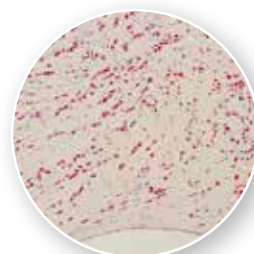
- ▶ Control tissue with sensitive antigens should be cut freshly or stored in a dry closed box in a fridge.
- ▶ All other control tissue could be stored in dry conditions at room temperature.



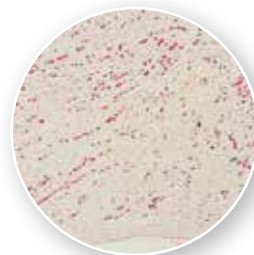
ER Immunohistochemistry:
Tissue section prepared freshly



ER Immunohistochemistry: Tissue section stored
at 4°C in a dry box for 5 month



ER Immunohistochemistry: Tissue section
stored at 22°C in a dry box for 5 month



ER Immunohistochemistry: Tissue section
stored at 37°C in a dry box for 5 month



ER Immunohistochemistry: Tissue section
stored at 22°C in a humid chamber for 5 month

Cytokeratin Pan Plus

A new cocktail for the detection of cytokeratins

Detection of cytokeratins with a broad spectrum ("pan"-) antibody allows for the staining of epithelial cells in normal and abnormal tissues. It is especially useful in characterisation of metastases with unknown origin as it distinguishes poorly differentiated epithelial carcinomas from non-epithelial malignancies.

The well established clone KL-1, which was an excellent tool for this differential diagnostic, is no

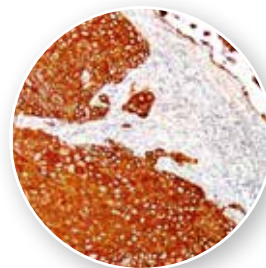
longer available on the market. Zytomed Systems developed a new cytokeratin cocktail comprising of the approved clones AE1, AE3 and 5D3.

The antibody of clone AE1 detects the acidic cytokeratins 10, 15, 16 and 19. The antibody derived from clone AE3 detects all basic cytokeratins, i.e. CK1 to 8. Clone 5D3 detects cytokeratins 8 and 18. This unique combination makes Cytokeratin Pan Plus an excellent alternative to Pan Cytokeratin clone KL-1.

► Cytokeratin fact box

Cytokeratins (CK) are intermediate filaments that constitute the cytoskeletal structure of virtually all epithelial but also of some non-epithelial cells. According to R. Moll they are divided into Type I (acidic cytokeratins, CK9 to 20) and Type II (basic cytokeratins, CK1 to 8) cytokeratins. Each Type I cytokeratin is co-expressed with a Type II cytokeratin inside a single cell. Hence, it follows that all epithelial cells contain at least two different cytokeratins. Only CK19 is expressed unpaired.

Moll R et al. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11-24, 1982



Cytokeratin staining on squamous cell carcinoma of the lung (MSK098-05)



Cytokeratin staining on colon carcinoma (MSK098-05)

► Product information

Description	Reactivity	CE/IVD	Pre-treatment	Dilution	Volume	Cat. No
Cytokeratin Pan Plus Clone: AE1, AE3, 5D3 Host: Mouse	Human, Mouse, Rat	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG098
				1:50 – 1:100	0.5 ml	MSK098-05

contact

ZYTOMED SYSTEMS GmbH
Anhaltinerstraße 16
14163 Berlin | Germany
Fon +49 30 804 984 990
Fax +49 30 804 984 999
info@zytomed-systems.de
www.zytomed-systems.com



Your local contact: