Zyto-Facts 1-2016

News for Pathology and Immunohistochemistry

ZYTOMED SYSTEMS

Editorial

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The primary site of a tumor is not known in about 3 % to 5 % of all cancer diagnoses. Patients, whose primary site could be identified, benefit from directed therapies and have improved outcome. Immunohistochemistry is widely used for differential diagnosis in addition to clinical, radiographic or classical histology methods.
Along with CDX-2, which was presented in the last issue of Zytofacts, recent publications have additionally described two new markers to identify colorectal origin: SATB2 and Cadherin 17. Furthermore, we discuss the diagnostic use of GATA-3 in triple negative mamma carcinoma.
Using positive control tissue is the key to sensitive, specific and reproducible immunohistochemistry. In our "focus: lab work" section you will find information describing how to find the right one.

Enjoy reading!
Karl-Georg Lintermann

+++ Newsflash +++ Newsflash +++

- **39es Assises de Pathologie Tour**
  26-27 May 2016
  Centre International de Congrès de Tours, Tour, France
- **29e Congrès de l'Association Française d'Histotechnologie**
  9-10 June 2016
  Institut National de la Transfusion Sanguine, Paris, France
- **Nottingham Pathology 2016**
  28 June–1 July 2016
  East Midlands Conference Centre, University of Nottingham, Nottingham, UK
- **ASCP 2016 Annual Meeting Pathology and Lab Medicine: Today, Tomorrow & Beyond**
  14-16 Sep 2016
  Mandala Bay, Las Vegas, NV, USA
- **XXXI International Congress of the International Academy of Pathology and the 28th Congress of the European Society of Pathology**
  25–29 Sep 2016
  Congress Centrum Ost Kölnmesse, Cologne, Germany
- **Carrefour Pathologie 2016**
  7–11 November 2016
  Palais des congrès, Paris, France
- **Herbsttagung 2016 der ÖGPath/IAP Austria**
  10–12 Nov 2016
  Tech Gate Vienna, Vienna, Austria
- **Medica 2016**
  14–17 Nov 2016
  Messe Düsseldorf, Düsseldorf, Germany

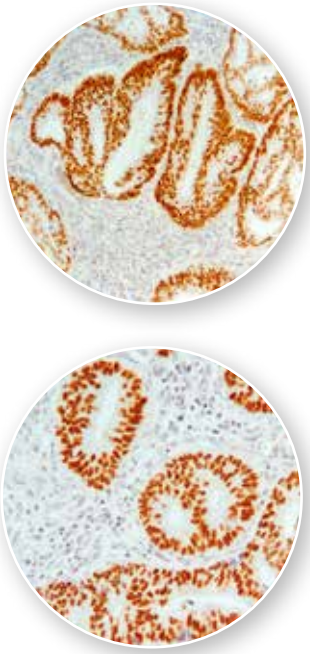
Two New Marker for Colon Carcinoma

SATB2 and Cadherin 17

Immunohistochemistry is a valuable tool for diagnosing a tumor metastasis if the primary is not known. Cytokeratin 7 (CK7) and Cytokeratin 20 (CK20) have been most widely used to predict the primary site especially if a colorectal carcinoma (CRC) is suspected. However, cases that do not show the typical CK7-/CK20+ immunophenotype are encountered frequently. A recent study recommends utilizing SATB2 and Cadherin 17 to identify or rule out a cancer of colorectal origin [1].

SATB2

Special AT-Rich Sequence-Binding Protein 2 (SATB2) is a DNA binding protein involved in transcription regulation and chromatin remodelling. The protein is selectively expressed in glandular cells of the lower gastrointestinal tract and expression is retained in a large majority of primary and metastatic CRCs [1].
SATB2 in combination with CK20 can identify more than 95 % of all colorectal carcinomas including poorly differentiated CRCs [2]. Lin *et al.* propose a panel of MLH1, Cadherin 17, and SATB2 when working on an unknown primary tumor, especially in an elderly patient with a CK7−/CK20− carcinoma [3].
In addition SATB2 can be helpful in identifying neuroendocrine neoplasms/carcinomas of the left colon and rectum because SATB2 is usually negative in other neuroendocrine neoplasms of the GI tract, pancreas, and lung [4]. Finally, recent publications described SATB2 as a sensitive marker for tumors with osteoblastic differentiation [5].



SATB2 staining on colon carcinoma

Product information

Description	Reactivity	CE/IVD	Pre-treatment	Dilution	Volume	Cat. No.
SATB2 Clone: SATBA4B10 Host: Mouse	Human, Mouse, Rat	-	HIER in EDTA pH 9.0	1:50 – 1:100	0.5 ml	MSK101-05
Cadherin 17 Clone: 1H3 Host: Mouse	Human	✓	HIER in EDTA pH 9.0	Ready-to-use	6 ml	MSG105
				1:100-1:200	0.5 ml	MSK105-05
CDX-2 Clone: EPR2764Y Host: Rabbit	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	RBG019
					16 ml	BRB028
				1:100	0.5 ml	RBK019-05
					1 ml	RBK019
Cytokeratin 7 Clone: OV-TL 12/30 Host: Mouse	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG032
					16 ml	BMS030
				1:100-1:200	0.5 ml	MSK032-05
					1 ml	MSK032
Cytokeratin 20 Clone: Ks20.8 Host: Mouse	Human	✓	Pepsin or Trypsin	Ready-to-use	16 ml	BMS037
Cytokeratin 20 Clone: polyclonal Host: Rabbit	Human	-	HIER in Citrate pH 6.0	Ready-to-use	7 ml	503-16441
				1:200	1 ml	503-16444

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[2] Magnusson K *et al.* SATB2 in combination with cytokeratin 20 identifies over 95 % of all colorectal carcinomas. Am J Surg Pathol 35:937-948, 2011

[3] Lin F *et al.* Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med 138:1015-1026, 2014

[4] Li Z *et al.* SATB2 is a highly sensitive marker for hindgut well-differentiated neuroendocrine tumors. Mod Pathol 26(suppl 2):164A, 2013

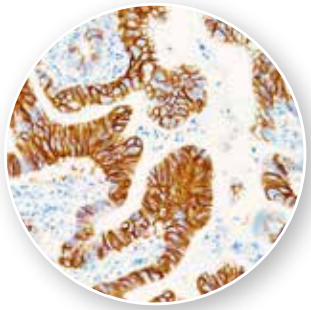
[5] Ordóñez NG. SATB2 is a novel marker of osteoblastic differentiation and colorectal adenocarcinoma. Adv Anat Pathol 21:63-67, 2014

Cadherin 17

Cadherin 17, also known as liver-intestinal Cadherin (LI-Cadherin) or CDH17, is a non-classical member of the Cadherin superfamily [1].

Cadherin 17 is involved in tumor invasion and metastasis and is expressed in the epithelial cells of embryonic and adult small intestine, colon, and pancreatic ducts. It is also frequently expressed in adenocarcinomas of stomach, colon, and pancreas. It has been shown that Cadherin 17 is a more sensitive marker than CDX2 in identifying adenocarcinomas of the colon, esophagus and pancreas [2–5]. Most CDX2 and CK20 negative medullary colon carcinomas are Cadherin 17 positive [6]. Panarelli *et al.* found a sensitivity of 100 % (161/161) for colon carcinomas using Cadherin 17, clone 1H3 [2]. The same sensitivity was reported by Tacha and Zhou (99/99). In this study clone 1H3 had a sensitivity of 73 % for adenocarcinomas of the stomach whereas the sensitivity of antibodies against CDX2 and Cytokeratin 20 was significantly lower, 16 % and 28 % respectively [4].
Several authors reported a remarkable specificity of Cadherin 17 [2–6]. It is rarely detected in hepatocellular carcinoma and carcinomas of lung and ovary. Lin *et al.* obtained a Cadherin 17 positivity in only 3.3 % of carcinomas outside the digestive system (n= 1671).

Cadherin 17 staining on colon carcinoma



Cadherin 17 staining on gastric cancer

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[2] Panarelli NC *et al.* Tissue-specific cadherin CDH17 is a useful marker of gastrointestinal adenocarcinomas with higher sensitivity than CDX2. Am J Clin Pathol 138:211-222, 2012

[3] Ordóñez NG. Cadherin 17 is a novel diagnostic marker for adenocarcinomas of the digestive system. Adv Anat Pathol 21:131-137, 2014

[4] Tacha D, Zhou D. CDH17 is a highly specific marker and is a more sensitive marker than CDX2 and CK20 in colon cancers. Poster session presented at: CAP14. The Pathologists' Meeting; 2014 Sep 7-10; Chicago

[5] Su MC *et al.* Cadherin-17 is a useful diagnostic marker for adenocarcinomas of the digestive system. Mod Pathol 21:1379-1386, 2008

[6] Lin F *et al.* Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med 138:1015-1026, 2014

Suggested reading for the topic "Cancer of unknown primary"

Lin F and Liu H. Arch Pathol Lab Med 138:1583-1610, 2014

Immunohistochemistry in Undifferentiated Neoplasm/Tumor of Uncertain Origin

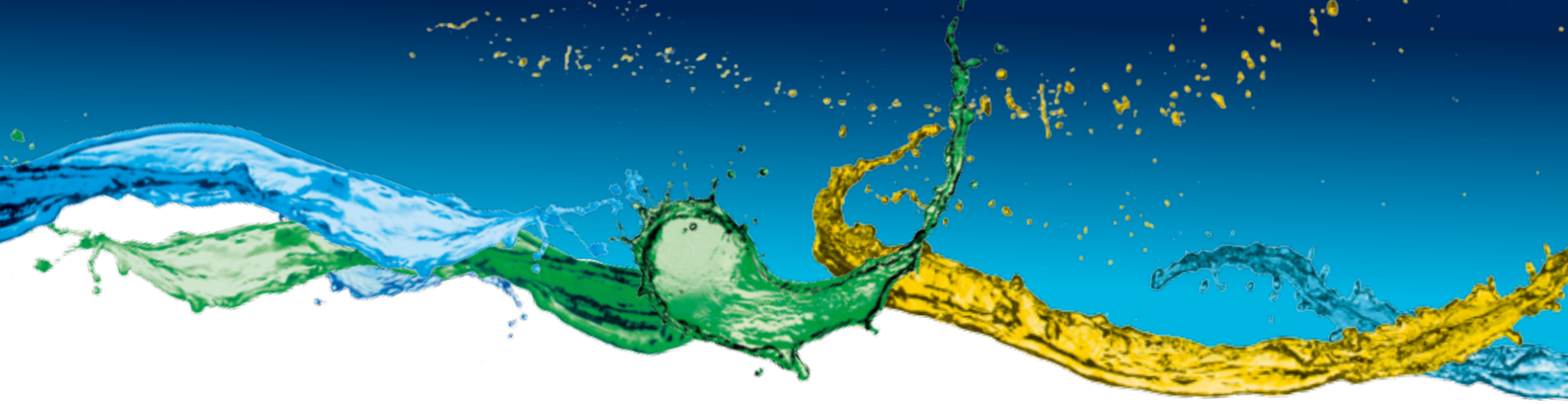
The authors present working algorithms for the identification and classification of undifferentiated neoplasia using immunohistochemistry and suggest diagnostic panels of antibodies for different entities. In this review Lin and Liu outline possible applications of recently described antibodies like SATB2, Cadherin 17 or Uroplakin II.

Some of the data originates from the „Handbook of Practical Immunohistochemistry: Frequently asked Questions." Lin et al., eds. New York, NY: Springer.

The paper is part of a comprehensive collection of reviews on immunohistochemistry published in two special editions of the ARCHIVES OF PATHOLOGY & LABORATORY MEDICINE.

Open access to the full texts under the following link:

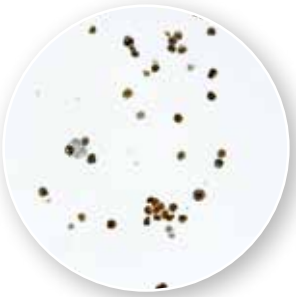
www.archivesofpathology.org/loi/arpa



How to Choose the Right Positive Control for Immunohistochemistry

Positive and negative controls help to verify whether the staining protocols have been performed correctly, and help determine day-to-day and operator-to-operator variations. A positive tissue control serves to document that proper staining (correct application and incubation times of reagents) has been performed and confirms that pre-analytic steps like fixation and pre-treatment have been carried out correctly.

Focus: lab work



ER immunostain on Cell Control Array Receptor

Her2 immunostain on Cell Control Array Receptor

What are the right properties of positive control tissue?

Of course the tissue must express the target antigen at a relevant, known and stable level. Very often (benign) tissue showing a very strong staining is recommended for positive control in datasheets for diagnostic antibodies. However, this tissue is only useful to determine if the antibody is working at all. False-negative results are likely to routinely arise considering that the antigen content of many tumors is significantly lower and will vary with the degree of tumor differentiation. Therefore it is advisable to use specimens of different low to medium expressing cases when establishing a control for a new antibody. In addition medium to low expressing control tissues are far better suited to detect decreasing sensitivity over a certain peroid of time. Prospective collecting of suitable tissues for positive controls will soon pay off! Keep in mind that tissue obtained from autopsies may already be partially degraded and may not be processed in the same way as your sample tissue.

Internal vs. external control

A positive internal control includes the respective antigen in the normal parts of the tissue sample in addition to the tissue elements to be evaluated. Many pathologists regard it as the perfect control because pre-analytics and pre-treatment are exactly in the same way in both parts of the tissue. However, in most accreditation processes a validated control is required. Since an internal control cannot be validated in advance, additional external controls are mandatory. If the test slide only contains tumor tissue, an internal control is also not an option.

On slide controls

Common practice is to use one positive control tissue on a separate slide per antibody in each staining run. However, this approach will not detect a missing or wrong reagent or inappropriate protocol on any other slides other than on the control slide itself. The solution to this problem is an on-slide control where the positive control tissue is placed on the same slide as the sample tissue. As an additional benefit it is very easy to prove that immunohistochemistry was carried out correctly if revalidation of the slide is necessary after storage.

Sensitive epitopes

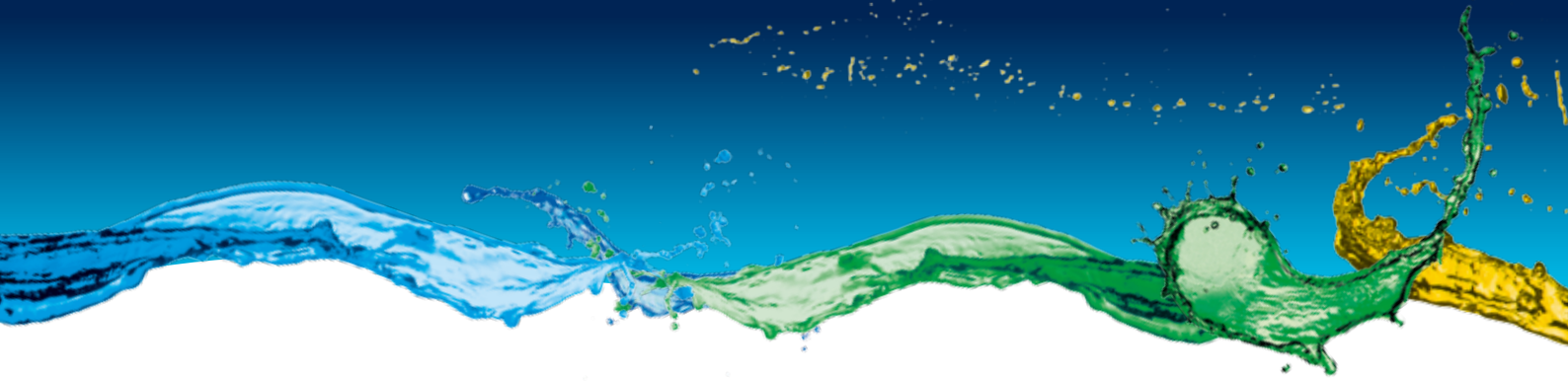
In most cases control tissue is cut and stored days or weeks before the immunostain. The previous Zytofacts "focus: labwork" section described proper storing of control slides. Note that not every antigen shows the same stability in sectioned tissue. Based on our experience TTF-1, Her2, MMR (mismatch Repair Proteins) and CD117 are particularly sensitive antigens and positive control slides for these markers should be stored dry and cool for not more than 2 weeks.

Tissue arrays and cell control arrays

Tissue microarray blocks containing selected tumors and/or normal tissues of various organs are extremely useful as positive and negative controls. NordiQC and Aalborg Sygehus DK, Department of Pathology propose a set of four tissue control arrays which cover most routinely used antibodies:
http://www.nordiqc.org/Techniques/Recommended_control_tissue.htm
Cell control arrays contain cores of cell lines expressing various types of antigens including pathogens. One advantage of commercially available cell arrays is the standardized level of antigen expression leading to block-to-block consistency of the control immunostaining.

Description	Amount	Cat No.
Cell Control Array Receptor	4 cores of mamma carcinoma cell lines	MB-CC REZ
Cell Control Array Virus	5 cores of virus infected cell lines	MB-CC VIR
Cell Control Array Bacteria plus Fungi	3 cores of different pathogens and one core of fungi	MB-CC BAC

All Cell Control Arrays are classified as for research-use-only.



Cytokeratin 5&14

A superior antibody cocktail for the diagnosis of the basal-like phenotype of breast carcinomas

This antibody cocktail reacts with cytokeratin 5 and cytokeratin 14. Basal cells of human epidermis express acidic cytokeratin 14 and basic cytokeratin 5. Cytokeratin 5 is closely related to cytokeratin 6. Both are expressed in basal cells of prostate, urothelium, vagina, squamous cell carcinomas of skin, tongue, epiglottis, rectal-anal region, and basal cell epithelioma. Cytokeratin 5 can be useful in the distinction of mesotheliomas from most adenocarcinomas whereas Cytokeratin 14 helps to distinguish stratified epithelial cells from simple epithelial cells. Cytokeratins 5 and 14 are very robust and reliable markers for squamous cell epithelia. Using antibody cocktails against these cytokeratins is helpful for the detection of basal cell layers and myoepithelia. Their use is well established for differential diagnosis of atypical proliferations and DCIS of the breast [1-4].

Advantages of CK5&14 over CK5&6

In breast cancer, CK14 is a major partner of CK5 and both of these filaments are associated with a basal-like phenotype. CK6, which is often used in combination with CK5 using the bi-specific antibody D5/16B4, is not expressed in normal breast tissue which speaks against its importance as a predominant marker of basal and progenitor cells of the mammary duct. CK5 clone XM26 (included in our CK5&14 antibody cocktail) has been shown to be superior to Cytokeratin 5&6 clone D5/16B4 [3].

NKX3-1

A new sensitive and specific marker of prostate origin

The diagnosis of metastatic carcinoma from uncertain primaries or poorly differentiated high-grade neoplasms involving the prostate and adjacent organs can be challenging, especially in the setting of limited cancer foci on a needle biopsy. In practice the histologic distinction between high-grade prostate cancer and infiltrating high-grade urothelial cancer has significant implications because both tumor entities require different treatments (i.e. hormone therapy for prostate cancer and chemotherapy for urothelial cancer). Although PSA and PSAP are highly selective markers for prostate, they have been shown to be only focally or weakly expressed in poorly differentiated prostatic carcinomas and prostate metastases [1]. On the other hand specific urothelial markers like Uroplakin III and Thrombomodulin are characterized by a low sensitivity especially in the metastatic setting [2].

NKX3-1 protein is a transcription factor encoded by the NK3 homeobox 1 gene located on chromosome 8. NKX3-1 is androgen-regulated and its expression is predominantly localized to the epithelium of normal prostate glands and prostate tumors.

Gurel *et al.* showed that the sensitivity in identifying metastatic prostatic adenocarcinomas was 98.6 % for NKX3-1 whereas the sensitivity of PSA staining was only 94.2 % [3]. Specificity reported by Gurel *et al.* was 99.7 % (n = 349).

Nuclear staining of NKX3-1 provides a distinct signal that is easy-to-interpret, similar to other transcription factors. Chuang *et al.* described NKX3-1 as a useful marker when high-grade prostate cancer is suspected but PSA staining is negative or equivocal [1]. The International Society of Urological Pathology recommends adding NKX3-1 to an antibody panel for the differential diagnosis of urothelial versus prostate carcinomas [4].

▶ Product information

Description	Reactivity	CE/IVD	Pre-treatment	Dilution	Volume	Cat. No.
Cytokeratin 5&14 Clone: XM26+LL002 Host: Mouse	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG106
					16 ml	BMS023
				1:50-1:100	0.5 ml	MSK106-05
					1 ml	MSK106
NKX3-1 Clone: polyclonal Host: Rabbit	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	RBG062
				1:50-1:100	0.5 ml	RBK062-05

Bibliography

[1] Boecker W *et al.* Common adult stem cells in the human breast give rise to glandular and myoepithelial cell lineages: a new cell biological concept. Lab Invest 82:737–745, 2002

[2] Hungermann D *et al.* Adenomyoepithelial tumours and myoepithelial carcinomas of the breast – a spectrum of monophasic and biphasic tumours dominated by immature myoepithelial cells. BMC Cancer 5:92, 2005

[3] Bhargava R *et al.* CK5 is more sensitive than CK5/6 in identifying the „basal-like" phenotype of breast carcinoma. Am J Clin Pathol 130:724-30, 2008.

[4] Bankfalvi A *et al.* Different proliferative activity of the glandular and myoepithelial lineages in benign proliferative and early malignant breast diseases. Modern Pathology 17: 1051–1061, 2004

NKX3-1 on prostate carcinoma

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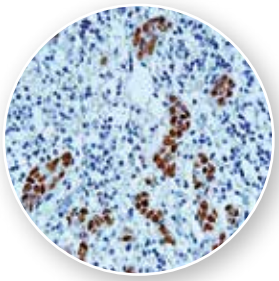
[1] Chuang A *et al.* Immunohistochemical differentiation of high-grade prostate carcinoma from urothelia carcinoma. AmJ Clin Pathol 31:1246-1255, 2007

[2] Chang A *et al.* Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus, and lung. Am J Surg Pathol. 36:1472-1476, 2012

[3] Gurel B *et al.* NKX3.1 as a marker of prostatic origin in metastatic tumors. Am J Surg Pathol, 2010

[4] Epstein JI *et al.* Best practices recommendations in the application of immunohistochemistry in the prostate: report from the International Society of Urologic Pathology consensus conference. Am J Surg Pathol 38:e6-e19, 2014



GATA-3 staining on breast carcinoma

GATA3 in Triple Negative Mamma Carcinoma

A new monoclonal antibody enhances specificity and sensitivity

GATA3 expression is associated with a better prognosis in patients with hormone receptor-positive/HER2-negative or triple-negative breast cancer (TNBC) [1,2]. In addition GATA3 is an important marker for the characterization of undifferentiated neoplasia with breast or bladder origin.

Identifying breast specific markers in TNBC can be challenging. Cimino-Mathews *et al.* showed that GATA3 expression could be detected more frequently than other breast markers in TNBC. Additionally, GATA3 expression is maintained between matched primary and metastatic carcinomas including ER-negative cases [3].

Krings *et al.* [4] reported a superior sensitivity of GATA3 compared to other breast markers like GCDFP-15 and Mammaglobin in TNBC. They also compared the immunohistochemical expression of two most commonly used GATA3 antibody clones, HG-31 and L50-823. L50-823 was positive in 66 % of TNBC whereas only 44 % stained positive with HG-31. The authors propose an immunopanel of GATA3, clone L50-832, Mammaglobin, and GCDFP15 for optimal diagnostic sensitivity in TNBC.

Product information

Description	Reactivity	CE/IVD	Pre-treatment	Dilution	Volume	Cat. No.
GATA-3 Clone: L50-823 Host: Mouse	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG100
					16 ml	BMS054
				1:50 – 200	0.5 ml	MSK100-05
					1 ml	MSK100
GCDFP-15 Clone: EP672Y Host: Rabbit	Human	-	HIER in Citrate pH 6.0	Ready-to-use	6 ml	RBG032
				1:25 – 1:100	0.5 ml	RBK032-05
Mammaglobin Clone: 31A5 Host: Rabbit	Human	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	RBG022
				1:100-1:500	0.5 ml	RBK022-05
Mammaglobin Clone: polyclonal Host: Rabbit	Human	-	HIER in Citrate pH 6.0	1:100-1:200	0.5 ml	RBK007

Bibliography

[1] Hisamatsu Y *et al.* Impact of GATA-3 and FOXA1 expression in patients with hormone receptor-positive/Her2-negative breast cancer. Breast Cancer 22:520-528, 2015

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