Accurate diagnoses as well as reliable prognostic and predictive information are the basis for making confident decision concerning the appropriate individual treatment of cancer patients. Tissue-based diagnostics like immunohistochemistry are becoming an increasingly important part of this process. High quality diagnostic reagents along with an in-depth knowledge of the characteristics and quality of different antibodies and clones as well as of protocols for tissue processing and staining are of essential importance. This issue includes:

- a comparison of the different specificities of “pan” or “broad-spectrum” cytokeratin antibodies
- a discussion of the advantages and disadvantages of two TTF-1 antibodies clones
- a description of how to process calcified tissue for optimal staining results

Enjoy reading this newsletter!
Karl-Georg Lintermann
Confident in differentiating mucinous ovarian and colorectal adenocarcinoma?
SATB2 is the marker of choice

SATB2 is a transcription factor involved in the regulation of osteoblastic and neuronal differentiation. Immunohistochemical studies have shown its specific expression in normal colorectal epithelium as well as in colorectal adenocarcinomas. Antibodies against SATB2 are recommended for inclusion in a panel of CK7, CK20 and CDX2 in order to identify the primary origin of poorly differentiated adenocarcinoma [1, 2].

Two recent studies address the value of SATB2 in the differential diagnosis of primary ovarian mucinous adenocarcinoma and metastasis of colorectal/appendical adenocarcinoma [3, 4]. Determination of the primary can be challenging because metastasis of extraovarian origin may mimic histological and immunohistochemical features of some primary ovarian mucinous tumors. In particular, several studies have shown a significant portion of primary ovarian adenocarcinomas to be positive with the colorectal marker CDX2 [5, 6]. In contrast, SATB2 immunoreactivity was not detected in ovarian adenocarcinomas lacking mature teratoma. SATB2 expression in ovarian metastasis could, however, be detected in 75% of colorectal adenocarcinomas and 86% of high and low grade appendiceal adenocarcinomas. No SATB2 expression was found in ovarian metastasis of pancreatic, gastric, gallbladder, or endocervical origin [3].

In another study, all 80 primary ovarian tumors not associated with cystic teratoma were SATB2 negative and all 20 metastatic ovarian tumors of the colon and appendix were positive for SATB2 [4]. In both studies, SATB2 was highly specific for colorectal and appendiceal metastases versus primary ovarian mucinous tumors with the exception of SATB2 expression found in teratomas of ovarian primaries. A new study investigated the diagnostic utility of SATB2 in metastatic Krukenberg tumors (MKTs), which is the third most frequent metastasis in the ovary. Immunohistochemical staining with SATB2 antibodies on 70 MKTs of various origins were performed. They describe SATB2 as a useful marker for determining the primary sites of MKTs of the ovary. In particular, SATB2 was the best marker for distinguishing MKT-metastatic adenocarcinomas ex goblet cell carcinoids [AdexGCC] from MKTs-stomach and MKTs-colorectum [7].

### Bibliography


### Product information

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SATB2 on colon
SATB2 on colon carcinoma
SATB2 on colon carcinoma
SATB2 on colon carcinoma
Cytokeratins (CK) are intermediate filaments that constitute the cytoskeletal structure of virtually all epithelial but also of some non-epithelial cells. They have been classified into basic (CK1 to 8) and acid (CK9 to 20) subfamilies and may also be distinguished by their molecular weights and their tissue distribution. Immunohistochemistry using antibodies against cytokeratins helps to classify undifferentiated neoplasia and metastases of unknown origin. Variations exist regarding sensitivity and specificity of the various broad spectrum or „pan” cytokeratin antibodies. The well-known broad spectrum CK antibody AE1 & AE3 is a mixture of two different clones of anti-cytokeratin monoclonal antibodies, AE1 and AE3. Clone AE1 detects the high molecular weight cytokeratins 10, 14, 15, and 16, as well as the low molecular weight cytokeratin 19. Clone AE3 detects the high molecular weight (HMW) cytokeratins 1, 2, 3, 4, 5, and 6, as well as the low molecular weight (LMW) cytokeratins 7 and 8. It is noteworthy that hepatocellular carcinomas are characteristically negative or only focally weakly positive with anti-cytokeratin AE1 & AE3. This is due to the fact that AE1 & AE3 does not recognize cytokeratin 18, which is expressed in most ductal and glandular epithelia. In addition, renal cell carcinomas and sometimes adrenal, prostate, and neuroendocrine carcinomas can be negative with AE1 & AE3 [1–4]. In order to overcome the low sensitivity of AE1 & AE3 in certain carcinomas, Zytomed Systems created the antibody Cytokeratin Pan Plus by adding the antibody clone 5D3 to the cocktail of clones AE1 and AE3. 5D3 detects CK8 and 18. Two other „Pan” clones were launched recently by Zytomed Systems, Cytokeratin Pan OSCAR and Cytokeratin Pan, clone KL1. A recent publication showed the superiority of clone OSCAR in detecting metaplastic breast carcinoma (MBC), especially spindle cell carcinomas [5]. Clone KL1 displays the broadest reactivity detecting cytokeratins 1, 2, 5, 6, 7, 8, 10, 11, 14, 16, 17, 18, and 19 [6].

Cytokeratin Pan Plus, KL1 and OSCAR react with a broad range of cytokeratins and therefore detect most liver and kidney carcinomas and are likely to detect more micrometastases in lymph nodes.

**Zytomed Systems’ antibodies against Cytokeratins**

**Description** | **Dilution** | **Volume** | **Cat. No.** | **Reactivity with Cytokeratins** | **Reactivity with Cytokeratins** |
|-----------------|-------------|------------|--------------|-------------------------------|-------------------------------|

**Basic (type II)**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |

**Acidic (type I)**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |

* Not available in all countries. Please contact your local distributor. | Reactivity according to Ordóñez (2013): 1, 2, 5, 6, 7, 8, 11, 14, 16, 17, 18

**Bibliography**


In routine histopathology, decalcification of bone and other calcified tissue is a prerequisite for the suitability of a specimen for the embedding procedure, for cutting, and for subsequent diagnostic techniques. Decalcification is usually carried out between fixation and routine processing to paraffin.

Three main types of decalcifying agents are alternatively based on strong mineral acids, weaker organic acids or those composed of chelating agents like EDTA (e.g. Titriplex).

EDTA works by capturing the calcium ions from mineralized tissue. The process is slow but very gentle and should be the method of choice for immunohistochemistry, in situ-hybridization or PCR. Decalcification is a standard procedure in many histopathology labs and is always a compromise between speed and optimal morphology of the tissue. Aspects of the process that are important for achieving high quality results are detailed in the following paragraphs.

EDTA is depleting in the decalcification process by binding with calcium: Instead of increasing the EDTA concentration of the decalcification buffer (which would affect tissue morphology), we recommend using a large volume and renewing it several times during the process. Increasing temperature during the process will speed up the procedure at the expense of tissue quality. According to our experiences, during incubation the temperature should not exceed 37 °C. Decalcification works better at high pH (up to pH 10) but tissue preservation will be not as good as with the standard pH 7.0. Gentle agitation may increase the decalcification rate slightly.

Determination of the end-point of decalcification is an important step. Incompletely decalcified tissue may be difficult to section (with damage to a microtome possibly occurring), whereas over-decalcification compromises morphology and impairs subsequent staining. X-ray and chemical testing using ammonium oxalate solutions are accurate but not routinely used in labs to prove complete decalcification. A physical test like bending the tissue or inserting a pin or razor blade into the specimen is not very precise and there is a risk of damaging the specimen, especially when done by inexperienced personnel. However, it’s quick, convenient, and sufficient in most cases.

When proceeding with standard embedding steps keep in mind that decalcified tissue could contain denser areas and thus requires a protocol that uses longer rather than shorter incubation times.

**Our lab recommends the following protocol for iliac crest punctures**

**Decalcification solution:**
- Prepare 10% Formalin by mixing 200 ml of 37% Formalin + 940 ml Aqua dest.
- Stir for 24h at 60 °C
- Adjust pH to 7.0 with 40% NaOH, store in the dark
- Dissolve 135 g EDTA (Titriplex III) in 900 ml 10% Formalin
- Incubate the specimen for about 8 h at 37 °C in decalcification solution.

**Suggested reading for the topic of SATB2 as a marker of colorectal origin**

Kyra B. Berg, MD; David F. Schaeffer, MD, FRCPC

**SATB2 as an Immunohistochemical Marker for Colorectal Adenocarcinoma:**
A Concise Review of Benefits and Pitfalls

From the Department of Pathology, University of British Columbia, Vancouver, Canada (Dr Berg) and the Department of Pathology, Vancouver General Hospital, Vancouver, British Columbia, Canada (Dr Schaeffer).

Berg and Schaeffer provide an overview of the current literature and summarize their practical experiences with SATB2. They describe SATB2 as a robust colorectal and appendiceal marker if used as part of a panel approach and as being more specific (but less sensitive) than CDX2 for an adenocarcinoma of unknown origin.

Open access to the full text under the following link: [https://doi.org/10.5858/arpa.2016-0243-RS](https://doi.org/10.5858/arpa.2016-0243-RS)
Thyroid Transcription factor 1 (TTF-1) a member of the NK2 family of transcription factors is a tissue specific transcription factor, expressed in the forebrain, thyroid and lung. TTF-1 plays a key role in morphogenesis of the lungs. Antibodies to TTF-1 are widely used in pathology diagnostics to identify lung and thyroid origin, not least because TTF-1 retains its high specificity and rather good sensitivity in metastatic and poorly differentiated tumors.

As a result of new therapies available for lung cancer, differentiation of pulmonary adenocarcinoma (ADC) vs squamous cell carcinomas of the lung (SqCC) has become more and more important in the clinical management of lung cancer patients. TTF-1 is the classical marker for ADC in this setting as part of a panel that includes p40, Cytokeratin 5 & 6 and Napsin A.

In addition to the well established clone 8G7G3/1, Zytomed Systems recently added clone SPT24 to their portfolio. Several studies have shown that both clones have different sensitivities for ADC and SqCC. In general, SPT24 is regarded as more sensitive for ADC whereas 8G7G3/1 is more specific [1, 2]. The higher sensitivity of clone SPT24 is reflected in the results of the external quality scheme NordicQC. In run 46, 2016, clone SPT24 is described as a „very robust and sensitive” marker for the demonstration of TTF-1 with 90% of all participants achieving good or optimal results. The number of false positive staining of SPT24 in SqCC can be significantly reduced by optimizing the titer and by using appropriate cut-offs [3]. In contrast to SPT24, clone 8G7G3/1 shows additional reactivity with hepatocytes and hepatocellular carcinomas (HCC) and therefore can be useful in distinguishing HCC from histological mimics. Immunohistochemistry using 8G7G3/1 in HCC results in a granular cytoplasmic stain pattern.

### Bibliography


**HSV I & II – An Antibody Cocktail against Herpes simplex**

*Herpes simplex* virus type I (HSV I, which mostly induces "cold sores") and *Herpes simplex* virus type II (HSV II, which mostly causes genital herpes) are two highly contagious members of the herpesvirus family that infect humans. About 3.7 billion people are infected with HSV I and 417 million are infected with HSV II under the age of 50 [1]. HSV infection commonly affects mucoepithelia or skin. HSV can infect a large variety of organs including brain, lung, liver, GI-tract and adrenal glands and lead to life-threatening conditions especially in cases involving immunosuppressed patients. A fast and reliable diagnosis increases a patient’s odds of survival. Zytomed Systems’ new antibody cocktail reacts with HSV type I and II specific antigens and with antigens common to HSV type I and II virus. It reacts with all the major glycoproteins present in the viral envelope as well as with at least one core protein [2, 3]. The Cell Control Array Virus (MB-CC VIR) containing cell lines infected with CMV, HSV type I and type II, EBV, and Polyomavirus/SV40 is perfectly suited as a positive control for immunohistochemistry using the HSV Cocktail. The small size of the control block sections allows patient material sections and control block sections to be mounted on the same slide.

### Bibliography


### Product description

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![Image of HSV I & II - An Antibody Cocktail against Herpes simplex](image-url)