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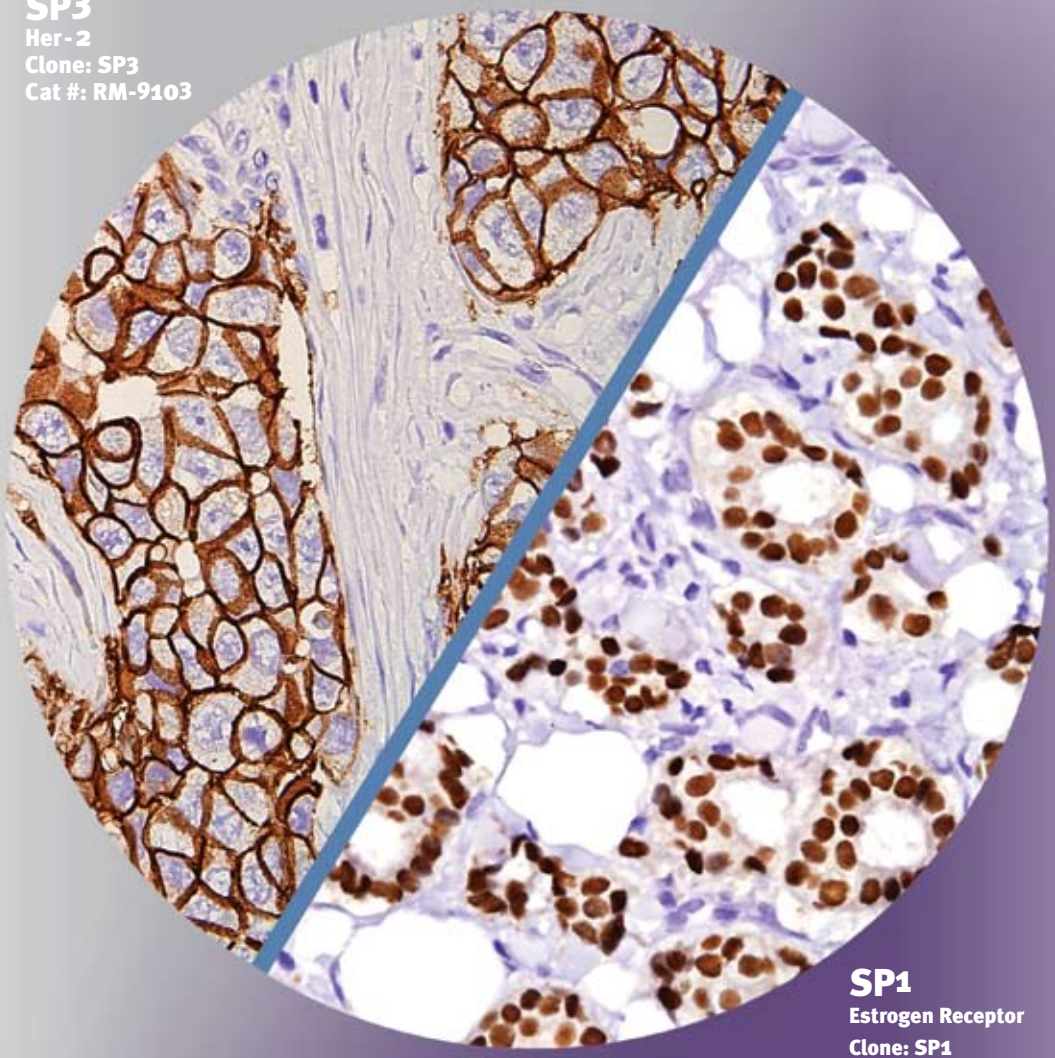
Volume 1, Issue 2



FEATURE ARTICLE

Thermo Scientific SP1 and SP3 Rabbit Monoclonal Antibodies:
Real World Advantages in Breast Cancer Analysis, page 2

Allen M. Gown, M.D.
Medical Director and Chief Pathologist
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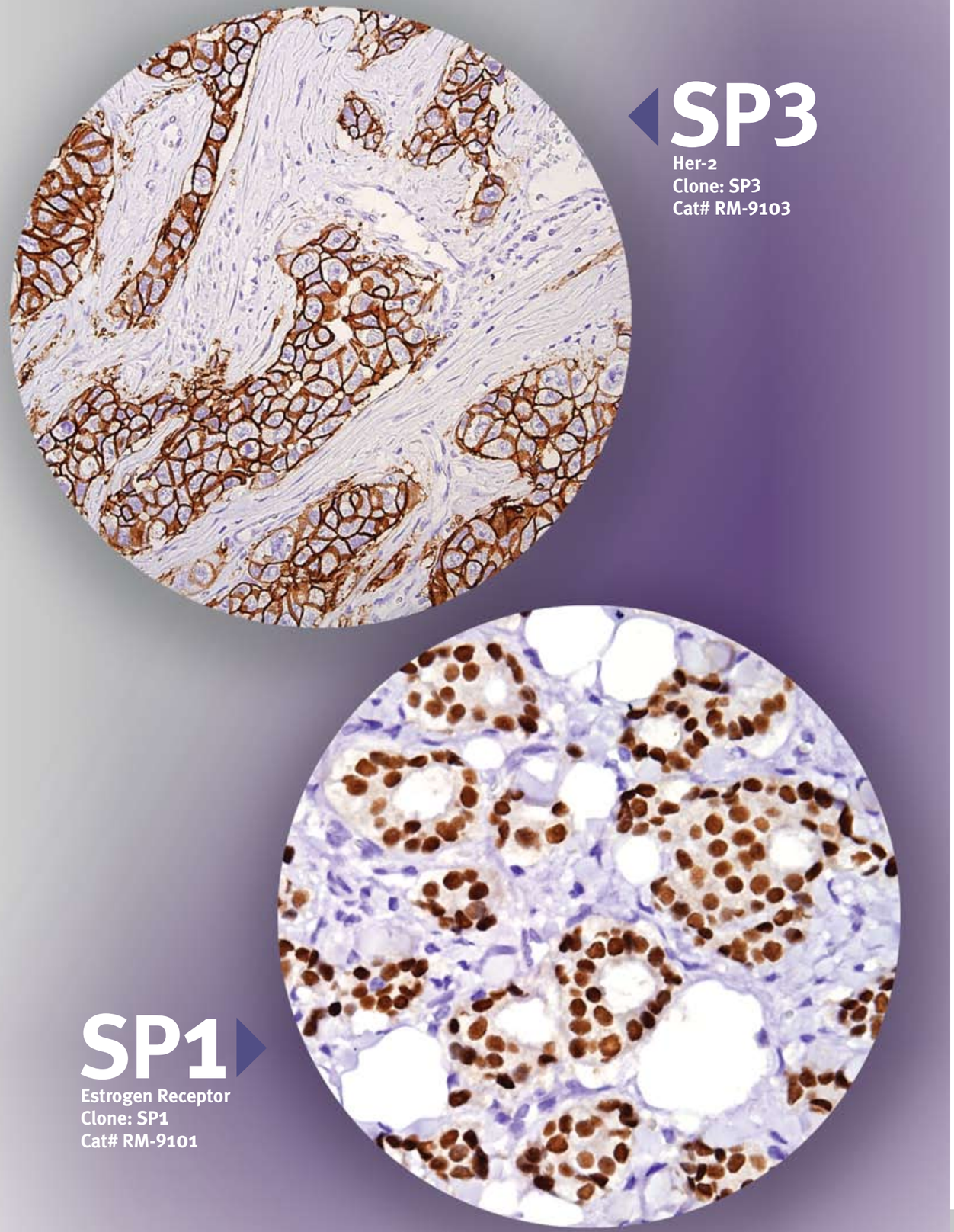


Thermo Scientific SP1 and SP3 Rabbit Monoclonal Antibodies: Real world advantages in breast cancer analysis

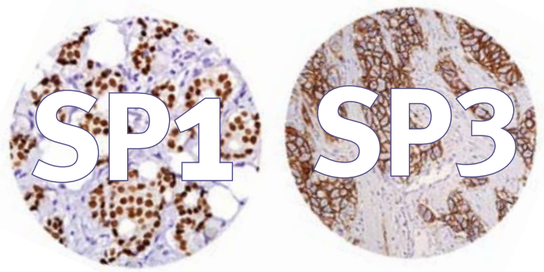
Allen M. Gown, M.D.
Medical Director and Chief Pathologist
PhenoPath Laboratories - Seattle, WA

Two Thermo Scientific rabbit monoclonal antibodies are poised to have a significant impact on the immunohistochemical analysis of prognostic and predictive breast cancer markers: Thermo Scientific SP1, a rabbit monoclonal antibody directed against the estrogen receptor alpha molecule, and target of tamoxifen; and Thermo Scientific SP3, a rabbit monoclonal antibody directed against the HER2 transmembrane receptor, the target of trastuzumab (Herceptin™).

SP1 has been demonstrated to have an eight fold higher affinity for the estrogen receptor compared with the 1D5 mouse monoclonal antibody that has been widely used in immunohistochemical analyses of breast cancer.[1] This higher affinity translates into a more robust immunohistochemical reagent, as was demonstrated in the paper published by Cheang et al,[2] describing a collaborative study performed by the British Columbia Cancer Agency and PhenoPath Laboratories. In this tissue microarray-based study of 4,150 patients in which determination of ER status with SP1 was compared with 1D5, with a median follow-up, of 12.4 years, SP1 was found in multivariate analyses to be a better independent prognostic factor than 1D5. Furthermore, determination of ER status using the SP1 antibody was more precise compared with the 1D5 antibody. The cohort, corresponding to 8% of the patients, who were SP1+ and 1D5-, i.e., who would have been classified as negative based on 1D5, were found to have a good outcome indicative of ER positive breast cancer. SP1-determined ER status also correlated better with ligand binding ER assay results. The study concluded that SP1 may represent an improved standard for ER assessment by immunohistochemistry in breast cancer.

More recent studies performed at PhenoPath Laboratories and presented this past spring at the USCAP meeting in Denver[3] document the potential advantages of SP3 as an immunohistochemical reagent in the assessment of HER2 status. In a series of 421 breast cancers analyzed for HER2 by immunohistochemistry, comparing the SP3 rabbit monoclonal antibody with a rabbit polyclonal antibody (Dako A0485), SP3 was found to be a more robust reagent, producing more consistent run-to-run immunostaining with fewer run failures. The study also showed that while both antibodies produced results that were greater than 95% concordant with those of FISH, the SP3 antibody was more "efficient" in yielding fewer 2+ cases.

SP1 and SP3 will undoubtedly be the subject of future studies, but the data to date suggest that both could well become the new gold standard for immunohistochemical analysis of breast cancer markers.



References:

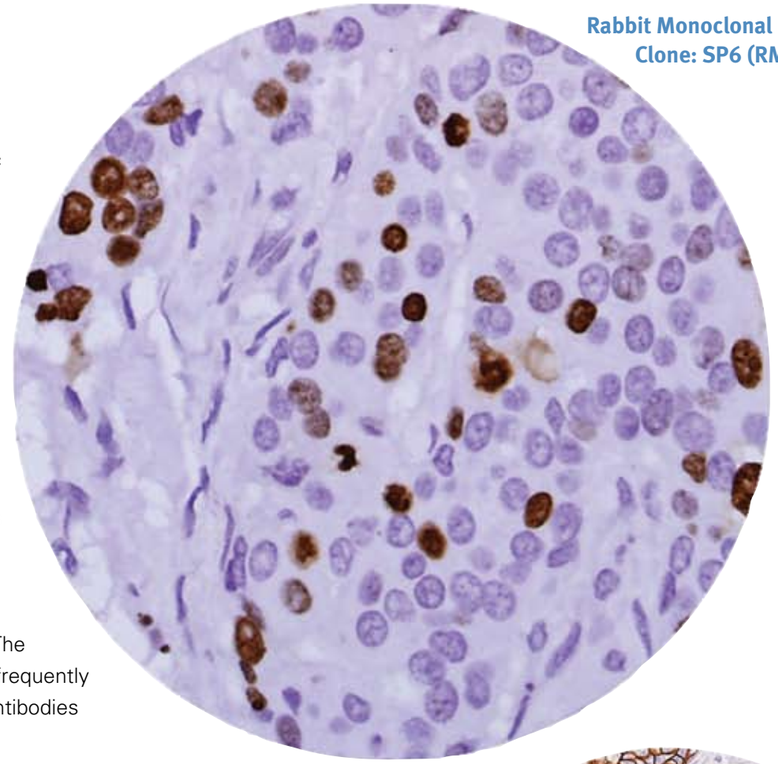
1. Huang Z., at el Appl Immunohistochem Mol Morphol. 2005; 13: 91-95.
2. Gown AM., at el. J Clin Oncol. 2006; 24: 5626-7.
3. Gown AM., at el. Mod Pathol. 2008.

Comprehensive IHC for Breast Pathology

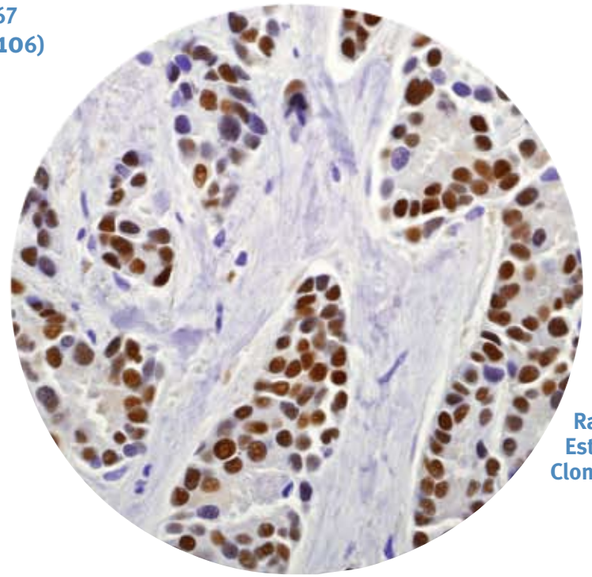
Tyler Liebig
Product Specialist
Thermo Fisher Scientific - Anatomical Pathology (Fremont, CA)

The ability of pathologists to accurately and confidently assess a patient's tumor is limited by the tests available from their laboratories. To fully understand all of the diagnostic and prognostic characteristics of a patient's tumor, pathologists must have a comprehensive array of sensitive, specific and accurate tests at their disposal. As research identifies useful new targets and better antibodies, laboratories may not always have the best tests available. To ensure laboratories have the most advanced technology for their antibody panels we have arranged our portfolio into panels based on tumor origin.

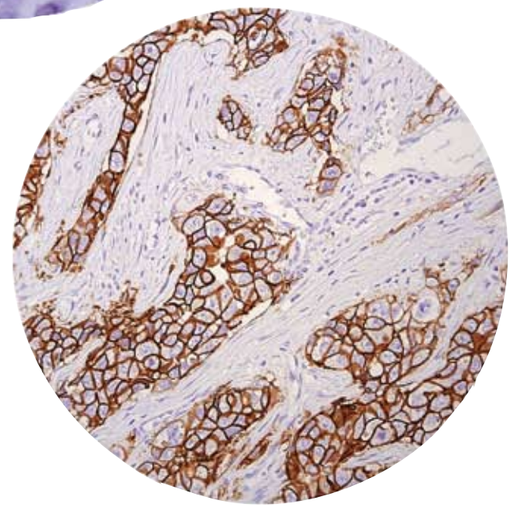
To ensure the best tests are always available, we are systematically reviewing our clinical portfolio by tumor origin, incorporating robust clones for each target. Building on a foundation of rabbit monoclonal antibodies, shown to be more sensitive and specific than current mouse monoclonal antibodies, we have finalized the first panel in our series.[1, 3, 5] We are pleased to announce the release of the Thermo Scientific Comprehensive Antibody Panel for breast pathology. The panel includes the latest additions to our rabbit monoclonal line such as Calponin-1, E-Cadherin, Beta-Catenin, and p53. Also included, is the soon to be released novel breast differentiation marker NY-BR-1. When compared to existing breast markers, NY-BR-1 shows a much higher specificity for cells originating in the breast (> 60%), with minimal cross reactivity to other cell types.[4,6] The panel still includes our original, frequently referenced rabbit monoclonal antibodies such as SP1, which is shown to be 8% more specific than 1D5 and a better predictor of survival.[1] Please see the included chart for the full list of anibodies in the comprehensive breast panel.



Rabbit Monoclonal Ki-67
Clone: SP6 (RM-9106)



Rabbit Monoclonal
Estrogen Receptor
Clone: SP1 (RM-9101)



Rabbit Monoclonal Her-2
Clone: SP3 (RM-9103)

We are excited about the opportunity our antibody panels provide clinical laboratories to refine and reinforce their IHC assessment and provide better results for their patients. Future launches of antibody panels include prostate, colon, lymphoma and differentiation markers. For more information about the breast panel and future launches visit our web site at www.thermo.com/labvision.

Antibody Name	RabMab	Clone	Cat #	Description	Pred.	Diag.	Ther.
ER	•	SP1	RM-9101	8% more sensitive than 1D5.[1]	•	•	•
PR	•	SP2	RM-9102	Specific and Sensitive.[2]	•	•	•
Her-2	•	SP3	RM-9103	Prognostic marker with clear results.[3]	•		•
Ki-67	•	SP6	RM-9106	Useful for determining tumor proliferation rates.	•		
E-Cadherin	•	EP700Y	RM-2100	Labels most ductal breast ca positive and most lobular breast ca negative.		•	
Keratin HMW		34betaE12	MS-1447	Labels most ductal breast ca positive and most lobular breast ca negative.		•	
Beta-Catenin	•	E247	RM-2101	Low expression may correlate with a poor outcome.	•	•	
Mammaglobin		304-1A5	MS-1919	Mammary marker and elevated expression may indicate a less aggressive tumor.	•	•	
GCDFP-15		23A3	MS-1170	Useful for identifying undifferentiated breast ca.		•	
NY-BR-1 Coming Soon!		NY-BR-1#2	MS-1932	Useful for identifying undifferentiated breast ca[4].		•	
Myosin Heavy Chain		SMMS-1	MS-1177	Labels myoepithelial cells. Used to examine invasiveness.		•	
Calponin-1	•	EP798Y	RM-2102	Labels myoepithelial cells. Used to examine invasiveness.		•	
Androgen Receptor		Poly	RB-9030	Useful for identifying undifferentiated breast ca.		•	
Keratin 5/6		D5/16	MS-1814	Myoepithelial marker, useful for determining invasiveness.		•	
Keratin 8/18		5D3	MS-743	Assists in the identification of adenocarcinoma.		•	
BCL-2		100/D5	MS-123	Overexpression in breast tumors can indicate poor prognosis.	•		
EGFR		EGFR.113	MS-1868	Overexpression may indicate a more virulent tumor.	•		
p53	•	Y5	RM-2103	Tumor suppressor with possible prognostic implications.	•		
Topoisomerase II alpha		Ki-S1	MS-1819	Only present in proliferating cells and is the target of several chemotherapies.	•		
Cathepsin D		Poly	RB-9051	Potential prognostic indicator.	•		
Cyclin D1	•	SP4	RM-9104	Overexpression in breast ca can indicate malignant transformation.	•		

References:

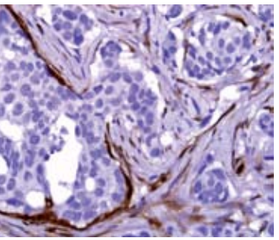
1. Cheang MC, et al. J Clin Oncol. 2006; 24(36): 5626-8.
2. Huang Z., et al. Appl Immunohistochem Mol Morphol. 2006; 14(2): 229-33.
3. Ricardo SA, et al. J Clin Pathol. 2006; 60(9): 1001-5.
4. Theurillat P., et al. Int J Cancer. 2008; 122(7): 1585-91.
5. Rossi S., et al. Am J Clin Pathol. 2005; 124(2): 295-302.
6. Theurillat P., et al. Clin Cancer Res. 2006; 12(9): 2745-51.

New Antibodies to Strengthen Your Routine Clinical Diagnostic Portfolio

Featuring Rabbit Monoclonal Calponin-1 (RM-2102) and E-Cadherin (RM-2100)

NEW

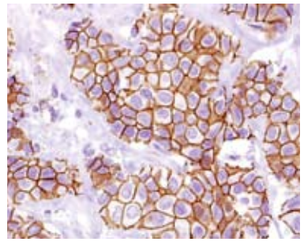
Rabbit Monoclonal Antibody Calponin-1 (RM-2102) Clone: EP798Y



Calponin, a thin filament-associated protein is implicated in the regulation and modulation of smooth muscle contraction. It is capable of binding to actin, calmodulin, troponin C and tropomyosin. Calponin is expressed in smooth muscle and tissues containing significant amounts of smooth muscle. Two isoforms of calponin exist whose molecular weights are 34kDa and 29kDa. Expression of the 29kDa form is primarily restricted to muscle of the urogenital tract. The expression of calponin has also been demonstrated in myoepithelial cells from benign and malignant breast lesions. It stains smooth muscle, myoepithelial cells, myofibroblasts, keratinocytes and nerve fibers. It identifies myoepithelial cells in breast lesions, and helps differentiate breast collagenous spherulosis (positive) from adenoid cystic carcinoma. Adenoid cystic carcinoma in salivary gland tumors is calponin positive. Calponin is used to differentiate non-invasive from invasive breast cancer.

NEW

Rabbit Monoclonal Antibody E-Cadherin (RM-2100) Clone: EP700Y



E-cadherin (uvomorulin, cell-CAM120/80) is a calcium-dependent cell adhesion molecule expressed predominantly in epithelial tissues. It plays an important role in the growth and development of cells via the mechanisms of control of tissue architecture and the maintenance of tissue integrity. Numerous studies have demonstrated that reduction and/or loss of E-cadherin expression in carcinomas correlates positively with the potential of these tumors for invasion and metastasis. E-cadherin is used in distinguishing lobular carcinoma in-situ (LCIS) from ductal carcinoma in-situ (DCIS) and that of invasive lobular carcinoma (ILC) from invasive ductal carcinoma (IDC) in indeterminate cases.

Expand your portfolio with more new Thermo Scientific antibodies.

Antibody	Clone	RabMab	Cat #	Description
Beta-Catenin	E247	•	RM-2101	Low expression may correlate with a poor outcome.
P53	Y5	•	RM-2103	Tumor suppressor with possible prognostic implications.
NY-BR-1 COMING SOON!	NY-BR-1#2		MS-1932	Useful for identifying metastatic breast ca.
Dynamin 1* COMING SOON!			MS-1931	Study of neuron and synaptic vesicle release.

*Research Use Only

Suggested References:

1. Douglas—Jones A., at el. Histopathology. 2005; 47(2): 202-8.
2. Jacobs TW, at el. Am J Surg Pathol. 2001; 25(2): 229-36.
3. Dolled-Filhart M., at el. Cancer Res. 1996; 66: 5487-94.
4. Royds JA, Iacopetta B. Cell Death Differ. 2006; 13(6): 1017-26.
5. Theurillat JP., at el. Clin Cancer Res. 2006; 12(9): 2745-51.
6. McClure SJ, Robinson PJ. Mol Membr Biol. 1996; 13(4): 189-215.

Towards Standardization in Immunohistochemistry

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President, Ancillary Pathways – Miami, FL

It is somewhat ironic that two adjacent components of the pathology laboratory, namely anatomic pathology (AP) and clinical pathology (CP), have been operating under different standards, for decades, at least until present time. Those of us who had combined pathology training in both disciplines are aware of this reality. In the clinical laboratory, testing of blood and body fluid samples is performed within very rigid and standardized conditions. Routinely, controls are evaluated alongside patients' samples. Accuracy and precision of each test are constantly being monitored to watch for the earliest drifts in the assay in question. Instruments and reagents are monitored in a similar fashion.

Cross the hallway and enter the AP lab, and you are likely to witness a more relaxed environment. Surgical specimen and biopsies are subjected to variable ischemic time, variable fixation time, variable thickness of tissue blocks, variable half life of fixative, variable types of fixatives, variable types of tissue processors, variable solutions in the processor, variable temperatures of different processor components, variable types of embedding paraffin, variable types of charged slides suitable for immunohistochemistry (IHC), variable drying times, variable oven temperatures, variable deparaffinization methodology, variable pretreatment buffers, variable pretreatment devices, variable instrumentation, variable IHC detection sensitivities, variable "enhancing" reagents, variable types of chromogens, variable counterstains, variable mounting media and variable interpretation of scoring of the final IHC reaction product by the pathologist. In case the reader missed the count, the word "variable" was mentioned twenty-two times in this paragraph.

To begin with, there is an intrinsic difficulty in achieving standardized testing in IHC, imposed by the mere nature of surgical specimen: unlike blood or urine samples, it is unrealistic to 'reproduce' a tissue sample in case the original sample fails the test. This fact makes it more difficult to achieve standardization. One might legitimately ask: why is standardization needed in IHC? Isn't the entire interpretation of H&E stained slides is largely subjective and hasn't been standardized in decades? The answer is simple: The better IHC will evolve as a technology, the more oncologists will rely on it to obtain information to treat their cancer patients. In other words, the scope of clinical applications of IHC in the 'old days' has been largely limited to complementing H&E in answering diagnostic questions. Increasingly, however, the previous success of IHC as a reliable tool for estrogen receptor testing[1] has led to a plethora of IHC predictive assays. It is reasonable to assume that this trend will continue for the next 10 years and perhaps longer until other proteomic technologies can reliably replace IHC.

The next logical question is, "Is it possible to achieve standardization in IHC and how?" The answer, to the best of my knowledge, is "yes" but with difficulty and a lot of

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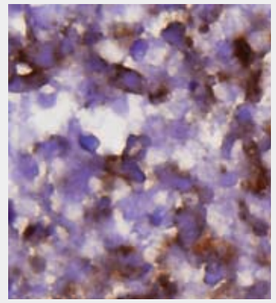
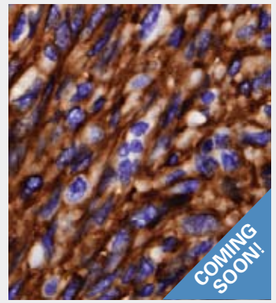
GastroIntestinal Stromal Tumor (GIST) stained with DOG-1.



Un-standardized tissue processing and IHC procedures causes poor cell morphology. Testing result can not be interpreted.



Standardized tissue processing and IHC procedures, preserve cell morphology and tissue integrity. It clearly shows cytoplasmic staining of DOG-1 (RM-9133).

effort. As suggested by my great mentor Clive Taylor, one of the pillars of this field, in order to accomplish some level of standardization in IHC, one must view and treat the IHC test as a 'total' test, meaning that each step of the testing component (pre-analytical, analytical, and post-analytical) must be tightly controlled and successfully duplicated in order to achieve a standard IHC test.[2] Recently, a number of official and ad-hoc meetings took place to address this very question. On January 2, 2008, pathologists and laboratory administrators in the US went to work and found out that from that day on they had to comply with minimum and maximum fixation requirements of breast cancer specimen in order to maintain certification of their laboratories.[3] For the very first time in the history of pathology in the US, a regulatory agency (College of American Pathologists) finally recognized the importance of imposing penalties on laboratories that do not closely monitor some of the pre-analytical factors in their labs, such as fixation time of breast cancers that will undergo HER2 testing. This, by far, is the first serious effort, albeit an imperfect one, toward standardization of IHC assays of HER2 testing.[4]

Two ad-hoc committee meetings have issued consensus statements in regard to general standardization of IHC[5] and specific standardization of IHC in estrogen receptor testing.

The main message from all of these organized efforts is two fold: a) to emphasize the importance of properly fixing and processing the tissue prior to performing the IHC test, and b) to stress the importance of standardizing all three components of the test, namely pre-analytical, analytical, and post-analytical.

There is a growing consensus that formaldehyde should remain the fixative of choice of specimen that require IHC testing until an alternate fixative can do the job. Also, in a reverse move toward standardization, all consensus opinions now agree that in order to "get the answer right versus getting it now," all IHC-tested clinical tissue samples must be fixed for a minimum fixation time of 6 hours, preferably 8-24 hours. The same concept favors conventional tissue processors, instead of rapid processors that utilize microwave and other technologies. Any deviation from these 'conventional' conditions will require that the medical director of the laboratory perform a side-by-side study to validate the 'deviating' component of the process.

Only well-documented analytical reagents (antibodies, detection systems, etc.), preferably those approved by the US-Food and Drug Administration as components of testing kits, should be used for clinical testing. Automation of the IHC assay is also a pre-requisite to standardized testing. Laboratories should, on a prospective basis, continue to monitor the rates of positive and negative results in order to detect any drift in the sensitivity of the assay. The use of different types of positive tissue controls is also mandatory to provide additional tools to monitor the assay.

In summary, standardization of IHC is not only possible; it is necessary. If we fail to accomplish it, this means that we will quickly have invalidated IHC as a useful assay that oncologists can rely on to determine treatment protocols for their patients. The first step in that direction involves adequate fixation and processing of tissue specimen.

References

1. Harvey JM, et al. J Clin Oncol. 1999 May; 17(5): 1474-81.
2. Taylor CR. Arch Pathol Lab Med. 2000 Jul; 124(7): 945-51
3. Wolff AC, et al. Arch Pathol Lab Med. 2007 Jan 1; 131(1):18
4. Yaziji H, Taylor CR. Appl Immunohistochem Mol Morphol. 2007 Sep; 15(3): 239-41
5. Goldstein NS, et al. Appl Immunohistochem Mol Morphol. 2007 Jun; 15(2):124-33.

Standardize Your Laboratory with Thermo Scientific Anatomical Pathology System Solutions

As Dr. Yaziji suggests, the first step towards the standardization of IHC involves adequate fixation and processing of tissue specimen. The Thermo Scientific STP 420D offers high quality routine, consistent, and repeatable procedures that standardizes the art of tissue processing. Optimize your laboratory with our comprehensive portfolio of Thermo Scientific Anatomical Pathology products, such as the ClearVue glass coverslipper, Autostainer and PTModule. When combined, all present a very compelling standardized system solution and deliver an optimal patient testing environment.

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A revolutionary instrument that combines fixation, dehydration, cleaning, and paraffin infiltration in one machine. The STP 420D when using Fast Flex technology (no microwaving) increases throughput and streamlines workflow by reducing processing times from 11-12 hours to less than 4.5 hours.

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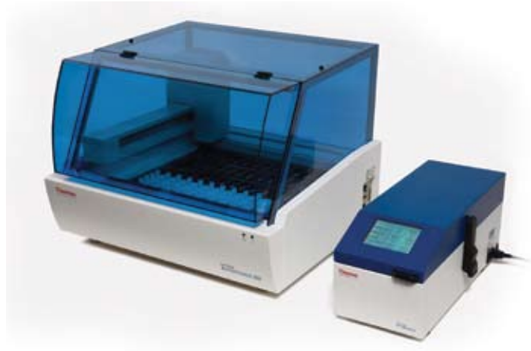
The PTModule is the benchmark of standardization in the antigen retrieval process. Combine with the Autostainer to streamline laboratory practices, optimize continuous workflow and deliver accurate, standardized and reliable results.



Pathology Corner:
An essential tool in the diagnosis of Malignant Mesothelioma

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Malignant Mesothelioma (MM) is an uncommon tumor arising from the serosal surfaces of the various body cavities, the most common; location being the pleura. It shows epithelial, sarcomatous, and biphasic mixed differentiation. Epithelial MM with its various histological patterns, needs to be differentiated from adenocarcinomas and other epithelial tumors. Sarcomatous MM, often being composed of solid sheets of pleomorphic spindle cells, will resemble a spindle cell or pleomorphic sarcoma. Immunohistochemistry (IHC) is the "sine qua none" to demonstrate mesothelial, epithelial or sarcomatous differentiation in the diagnosis of mesothelioma, that rendered obsolete many previously used tests. Current markers generally have very good sensitivity and specificity for the differential diagnosis of MM, adenocarcinomas of lung, and tumor of other origin. However, it yields a lower accuracy for the differential diagnosis of MM and other epithelial neoplasms, such as squamous cell carcinoma, and carcinoma of kidney, bladder, ovary, and other sites.[1]

In addition, MM epithelial-type can be very difficult to distinguish from reactive mesothelial hyperplasia with cytological atypia.[2] Suggested IHC panel for this includes keratin AE1/AE3, EMA, p53, and Desmin. Strong Desmin expression is indicative of a reactive process.

Mesothelial markers except Calretinin show very low sensitivity and specificity for the diagnosis of sarcomatous MM.[3] These technical limitations, along with garden variety histological patterns and wide range of differentiation, have promoted the use of antibody panels rather than the use of one or two markers for the diagnosis of MM.[4] Different experts have varying preferences as to how many antibodies (from a long list) to include in their differential diagnosis of MM. There is growing body of evidence about the need to establish evidence-based guidelines to determine which epithelial and mesothelial markers need to be included in the differential diagnosis of antibody panels.[5]

TABLE-1 shows the most useful mesothelial and epithelial markers available for the diagnosis of MM and its differential diagnosis from selected carcinomas and sarcomas, adopted from Marchevsky.[5]

Antibody	Epithelial MM, % postitive	Sarcomatous MM, % Positive	Adenocarcinoma, % Positive
Epithelial Marker			
pCEA (polyclonal carcinoembryonic antigen)	5	0	83
mCEA (monoclonal carcinoembryonic antigen)	3	---	81
Ber-Ep4	10	0	80
B72.3	7	0	80
CD15 (leu-M1)	7	0	72
MOC-31	7	0	93
TTF-1 (thyroid transcription factor)	Negative	0	72
Mesothelial Marker			
Cytokeratin 5/6	83	13	14.9
Calretinin	82	88	15
HBME-1	85	---	57
Thrombomodulin	61	13	20
WT-1	77	13	4
Mesonthelin	100	0	---

TABLE-2 indicates the distinction between benign mesothelial reactions and malignant mesothelioma, adopted from Marchevsky.[5]

Antibody	Benign Afypical Mesothelial Proliferations	Malignant Mesothelioma
Keratin AE1/AE3	+/-	+++
EMA	+/-	+++
Desmin	+++	+/-

** +/- indicates present in some tumor cells; +++, present in most tumor cells; and EMA, epithelial membrane antigen.*

Below are the most reliable Thermo Scientific Mesothelial and Epithelial markers. Please visit our website for more information **www.thermo.com/labvision.**

Antibody Name	Clone	Cat#
Calretinin	SP13	RM-9113
CK5/6	D5/16 B4	MS-1814
Wilm's Tumor protein (WT1)	Poly	RB-9267
Mesothelin	5B2	MS-1320
Mesothelioma	HBME-1	MS-1494
CEA	CD66e	RB-368
TTF-1	8G7G3/1	MS-699
CD-15	MMA	MS-1259
Keratin, Pan Ab-1	AE1/AE3	MS-343
TAG-72/CA 72-4 Ab-1	B72.3	MS-138

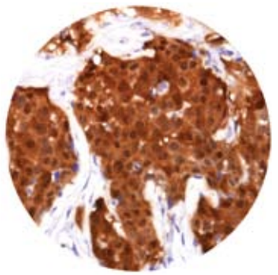

Figure 1.
Calretinin (RM-9113) Maligant mesotheiloma, epithelioid variant stained for Calretinin. The tumor cells exhibit strong nuclear and cytoplasmic staining.

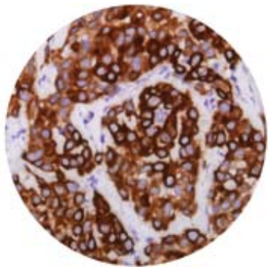

Figure 2.
Cytokeratin 5/6 (MS-1814) Maligant mesotheiloma, epithelioid variant stained for CK5/6. The tumor cells exhibit strong cytoplasmic staining.

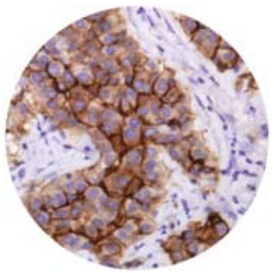

Figure 3.
Mesothelin (MS-1320) Maligant mesotheiloma, epithelioid variant stained for Mesothelin. The tumor cells exhibit strong membrane immunoreactivity.

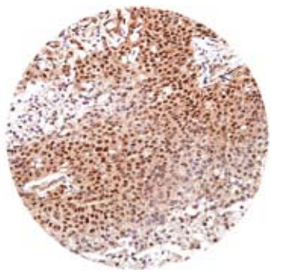

Figure 4.
Wilm's Tumor Protein (RB-9267) Maligant mesotheiloma, epithelioid variant stained for WT1. The tumor cells exhibit strong nuclear staining.

References:

1. Marchevsky AM, Wick MR. Appl Immunohistochem Mol Morphol. 2007;15:140–144.
2. Attanoos RL et al. Histopathology. 2003; 43: 231-38
3. Lucas DR, et al. Histopathology. 2003;42:270–279.
4. Yaziji H., et al. Mod Pathol. 2006;19:514–523.
5. Marchevsky AM, Arch Pathol Lab Med-Vol l32, March 2008

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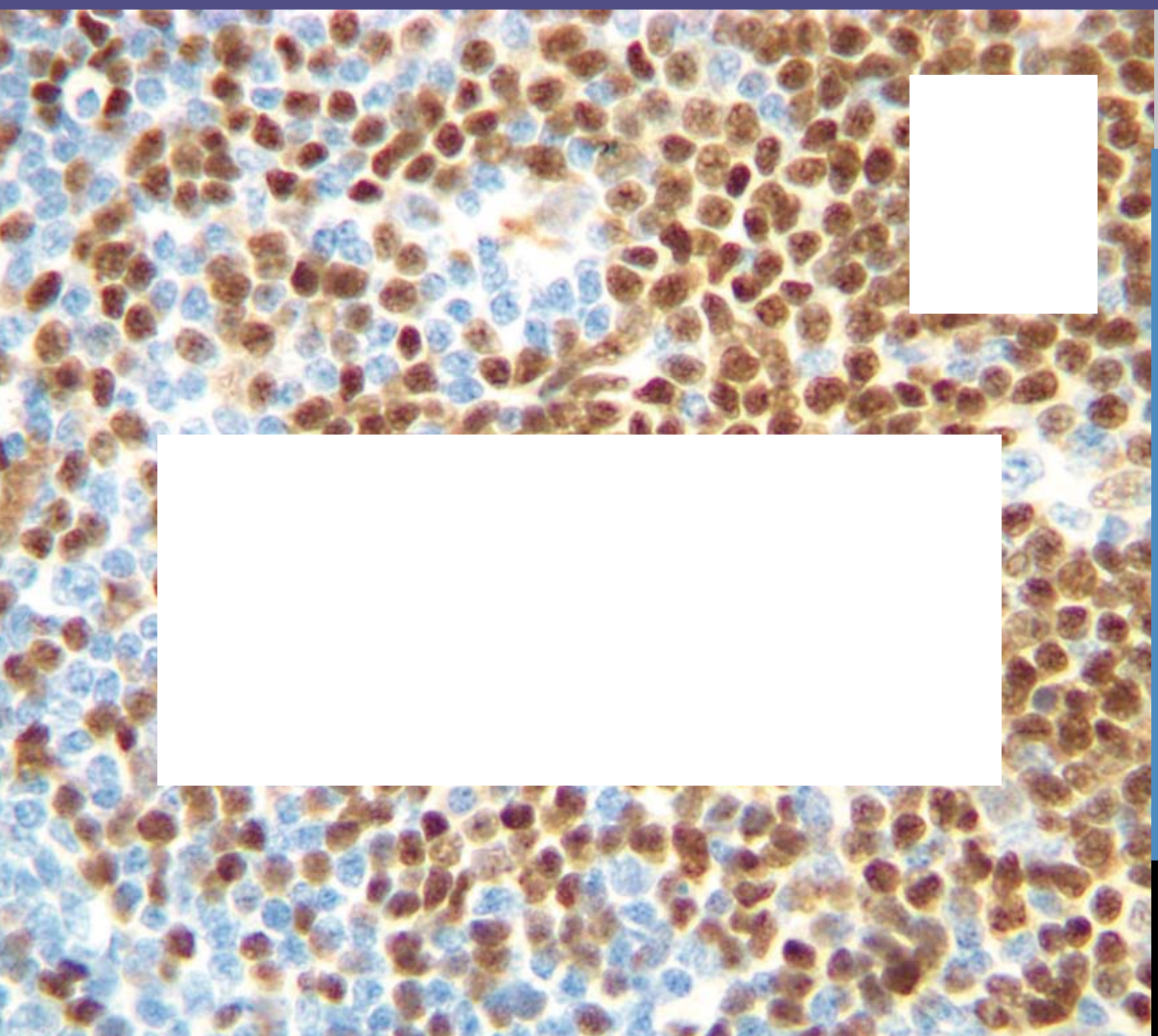

Mantle cell lymphoma stained with Cyclin D1 Clone SP4 (RM-9104)

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