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FEATURE ARTICLE

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The Potential of Peptide Analyte Control Slides in IHC

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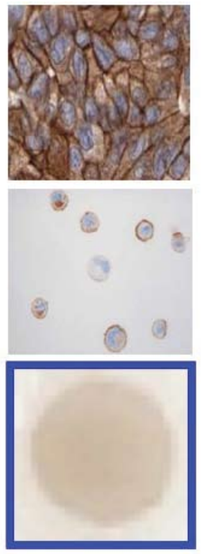
In recent years, the human epidermal growth factor receptor 2 (HER2) has become an important prognostic and predictive marker being regularly evaluated and triggering therapeutic options in the management of patients suffering from breast cancer.

Recent publication data from proficiency testing surveys indicates a double-digit rate of compromised results of HER2/neu immunohistochemistry tests. Although, the number of successful participating laboratories continuously increases, if stratifying for HER2 score, the evaluation of 2+ scored reference material appears to remain an area requiring improvement.

Recently, a study was performed within the proficiency survey of the College of American Pathologists utilizing a new concept of an external IHC control slide used in the analytical phase of the IHC staining procedure intended to detect issues in antigen retrieval, antibody and staining protocols. When both quantifying the peptide analyte controls and reviewing the sections, in approximately 20% of 109 study participants that returned these special slides, the staining was found to be suboptimal. Interestingly, 35% of the issues could be attributed to antigen retrieval, 20% to antibody or staining protocols, and 45% to a combination of the latter.

The concept of peptide analyte controls is based on short peptides that have been identified by phage display technology to be immunoreactive towards the antibody clone(s) of interest. Peptide epitopes are linear and most of them will have less than 30 amino acids. They simulate the portion of the native antigen to which an antibody binds, making it possible to use peptide epitopes as a control target for one or more antibody clones used in IHC.



Analytical Phase

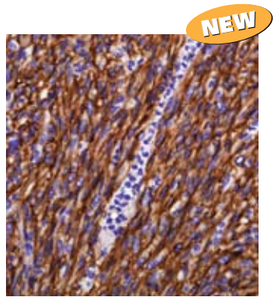
There are many advantages to using peptides epitopes: short peptides have the same specific immunoreactivity as the whole antigen; short peptides can be blended creating a specific target for several antibody clones and mapping for one or more biomarkers. Peptide epitopes do not degrade during, or after deparaffinization; they can be easily synthesized and therefore be produced in infinite quantities and in consistent quality. The dilution and the spotting of peptides onto a special slide is a highly controllable process that guarantees high accuracy and precision in manufacturing.

The current research work underway at Thermo Fisher Scientific will define the design of the first peptide analyte control slide commercially available as an external control specifically for the application of the biomarker HER2/neu in the analytical phase of the IHC staining procedure.

Reference:
S. Bogen et al. Arch Pathol Lab Med. 2008; 132:211–216.

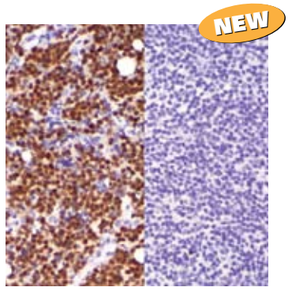
Exciting New Thermo Scientific Antibodies!

Featuring Rabbit Monoclonal DOG-1 (RM-9132) and PAX-5 (RM-9133)



Rabbit Monoclonal Antibody
DOG-1 (RM-9132)
Clone: SP31

DOG-1 is a cell surface protein of unknown function selectively expressed in GIST. The positive rate increases with the use of the DOG-1 antibody. DOG-1 identifies the vast majority of both c-Kit negative and PDGFRA mutated GIST cases that may still benefit from imatinib mesylate (Gleevec), an inhibitor of the kit tyrosin kinase. In addition, DOG-1 immunoreactivity is seen in fewer cases of mesenchymal, epithelial tumors and melanomas when compared with c-Kit. The use of this highly sensitive and specific novel marker will increase the accuracy of GIST diagnosis.



Rabbit Monoclonal Antibody
PAX-5 (RM-9133)
Clone: SP34

PAX-5 is a B-cell-specific activator protein (BSAP). In the early stages of B cell development, PAX-5 influences the expression of several B-cell-specific genes, such as CD19 and CD20. PAX-5 is expressed primarily in pro-, pre-, and mature B cells, but not in plasma cells. PAX-5 is expressed in most B-cell malignancies (pre-B and mature B cell lymphomas/ leukemias). Over 90% of cases are positive for PAX-5 in Hodgkin's lymphoma, lymphoplasmacytic lymphoma, Merkel cell and small cell carcinoma. T-cell lymphomas, myeloma/ plasmacytoma and carcinoid tumors are negative for PAX-5.

More new antibodies to further strengthen your diagnostic porfolio

Antibody	RabMab	Clone	Cat #	Description	Prognostic Factor	Diagnostic	Predictive Factor
CK 5	•	EP 160 1Y	RM 2106	Closely associated with CK 6. Plays an important role in the diagnosis of epithelioid mesothelioma, squamous ca, breast ca, and other types of ca.[1]		•	
CK 8	•	EP162 8Y	RM-2107	Identifies carcinoma of simple epithelium, including breast ducts, gallbladder, intestine, liver, pancreas, and prostate. Commonly paired with CK 18.		•	
CK 18	•	E431-1	RM-2108	Distinguishes simple epithelioid cells frequently expressed in various adenocarcinoma, including gastric and hepatocellular.[2] Commonly paired with CK 8.	•	•	
PAX-5	•	SP34	RM-1933	Extremely specific marker of the B lineage. Tremendous diagnostic benefit to include in the panel for investigation of undifferentiated neoplasms.[3]		•	
CD3 Epsilon	•	EP449E	RM-2109	Strongly recommended for the diagnosis of T-cell lymphomas.		•	
DOG-1	•	1.1	MS-1933	Differentiation marker for KIT-and PDGFRA-mutated GIST[6]. Has higher sensitivity and specificity for diagnosing GIST.		•	•
DOG-1	•	SP31	MS-9132	Differentiation marker for KIT-and PDGFRA-mutated GIST[6]. Has higher sensitivity and specificity for diagnosing GIST.		•	•

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Suggested Reference:

1. Socorro RP., et al. Clin Cancer Res. 2006; 12(5): 1533-39.
2. Kim MA, et al. Human Pathol. 2004; 35(5): 576-81.
3. Jensen KC, et al. Mod Pathol. 2007; 20(8); 871-7.
4. Paul PC, et al. Indian J Pathol Microbiol. 2007; 50(2): 279-83.
5. Espinosa I., et al. Am J Surg Pathol. 2008; 32: 210-18.
6. Robert W. et al. Am J Pathol. 2004; 165(5): 107-113.

Comprehensive Thermo Scientific Prostate Panel Now Available

Tyler Liebig
Associate Product Manager
Thermo Fisher Scientific – Anatomical Pathology (Kalamazoo, MI)

Other than skin cancer, prostate cancer is the most common form of cancer in men. The identification of prostate cancer and prostatic intraepithelial neoplasia often relies on limited amounts of patient sample. In order to accurately detect and differentiate prostate carcinoma, precise and reliable immunohistochemistry (IHC) tests are required. The comprehensive Thermo Scientific Prostate Panel is now available to provide the ideal tests for the assessment of prostate cancer.

The panel is built on a strong core of proven markers for the assessment of prostate cancer. Proven markers such as PSA, p504s and Keratin 34betaE12 are all included in the Thermo Scientific portfolio. In addition, several new rabbit monoclonal antibodies are now available. Many rabbit monoclonal antibodies continue to show superior results to currently available markers.[3]

Two new rabbit monoclonal antibodies included in the prostate panel are Prostate Specific Antigen (PSA Clone: EP1588Y) and Prostate Specific Membrane Antigen (PSMA Clone: SP29). Mouse monoclonal antibodies against PSA have been available for many years. It still is a very useful marker, but sensitivity is not ideal in all cases.[1] Specifically, PSA expression decreases in poorly differentiated prostatic carcinoma.[1] Inclusion of a PSMA stain, where metastatic prostate cancer is suspected, can help correct identification of a higher percentage of cases on the first pass[1,2]. It has been reported that PSMA is one of the better markers for labelling prostate cancers, especially those that show poor PSA staining[1] as shown in Fig. 2. In the same case Fig. 1 shows clearly positive PMSA staining using rabbit monoclonal antibody, clone SP29, from Thermo Scientific.

Thermo Scientific is dedicated to providing optimal tests in the interests of laboratories and patients. Utilizing these new antibodies, in combination with established antibodies from the comprehensive Thermo Scientific Prostate Panel ensures that every laboratory has the most advanced antibody technology available.

References:
1. Chuang AY., at et al. Am J Surg Pathol. 2007; 31(8):1246-55.
2. Mhawech-Fauceglia P., et al. Histopathology. 2007;50(4):472-83.
3. Rossi S., et al. Am J Clin Pathol. 2005; 124(2): 295-302

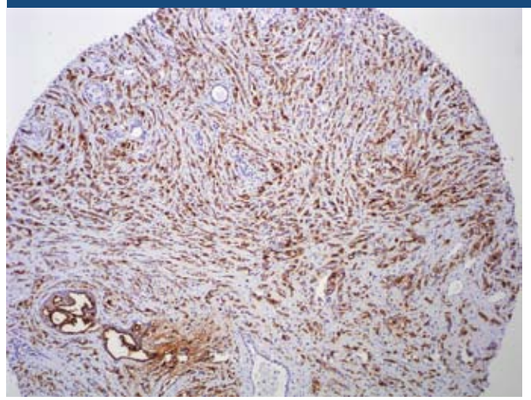

Figure 1: Positive PSMA Staining
Rabbit Monoclonal
Clone: SP29 (RM-9131)

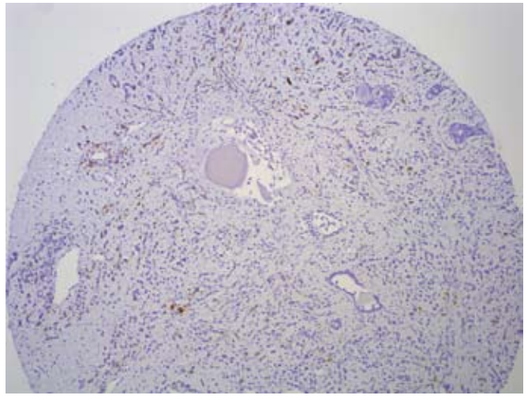

Figure 2: Weak expression of PSA Staining
Rabbit Monoclonal
Clone: EP1588Y (RM-2104)

Comprehensive Prostate Pathology

Antibody	RabMab	Clone	Cat#	Description	Prog.	Diag.	Ther.
PSA	•	EP11588Y	RM-2104	Sensitive and specific for tumors of prostatic origin.[1]		•	
PSMA	•	SP29	RM-9131	Useful for identifying neoplasms of prostatic origin. Increased expression has also been shown to correlate to meatstatic progression.		•	
PSAP		PASE/4LJ	MS-321	Useful for the identification of metastatic adenocarcinoma of the prostate.[1]		•	
p504S (AMACR)	•	13H4	RM-9130	Typically strongly positive in malignant prostate but weak or negative in benign lesions.[1]		•	
p63*		4A4	MS-1081	Differentiates glands by identification of basal cells.[1]			
Keratin HMW		34BetaE12	MS-1447	Basil Cell marker used to differentiate benign from malignant prostatic carcinoma.[1]		•	
Androgen Receptor		AR441	MS-443	Believed to play a critical role in the development of prostate cancer.		•	
Ki-67	•	SP6	RM-9106	Can serve as predictor of recurrence, progression and survival.[2]	•		
p53	•	Y5	RM-2103	Overexpression is associated with increased cellular proliferation and also indicates an adverse outcome after radiation therapy.[2]	•		
PTEN*		28H6	MS-1797	Low expression in prostate cancer indicates a poor prognosis.[3]	•		•
p27		Poly	RB-9019	Predictor of biochemical relapse.[4]	•		
E-Cadherin	•	EP700Y	RM-2100	Abnormal E-cadherin coupled with biochemical failure can indicate subclinical disemination.[5]	•		•
ESA/Ep-CAM		VU-1D9	MS-144	Generally overexpressed in prostate carcinoma.[6]	•		•
BCL-2		100/D5	MS-123	Bcl-2 in addition to PSA could identify patients that will benefit from chemotherapy.[7]	•		•
MUC-1		Poly	RB-9222	Shown to be an independent prognostic marker for prostate cancer death.	•		

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Table References:
1. Paner G, Luthringer D, Amin M. Arch Pathol Lab Med. 2008;132:1388-1396.
2. Moul JW. Eur Urol 1999;27:688.
3. McCall P, Witton CJ, Grimsley S, Nielsen KV, Edwards J. Br J Cancer. 2008 Oct 21;99(8):1296-301.
4. Ribal MJ, Fernandez PL, Lopez-Guillermo A, Farré X, Santos Y, Gibanel R, Cardesa A, Alcaraz A. Anticancer Res. 2003 Nov-Dec;23(6D):5101-6.
5. Ray ME, Mehra R, Sandler HM, Daignault S, Shah RB. J Urol. 2006 Oct;176(4 Pt 1):1409-14; discussion 1414.
6. Poczatek RB, Myers RB, Manne U, Oelschlager DK, Weiss HL, Bostwick DG, Grizzle WE. J Urol. 1999 Oct;162(4):1462-6.
7. Yoshino T, Shiina H, Urakami S, Kikuno N, Yoneda T, Shigeno K, Igawa M. Clin Cancer Res. 2006 Oct 15;12(20 Pt 1):6116-24.

Improvements in Thermo Scientific IHC Instrument Manufacturing

Sheila Dandy
Customer Care Manager
Thermo Fisher Scientific – Anatomical Pathology (Runcorn, UK)

In November 2008, the first 3 Thermo Scientific Autostainer 480S instruments were built and shipped from our manufacturing facility in Runcorn, UK. This does not sound remarkable in itself – the current design of Autostainer has been on the market since April 1996 – but until recently, these instruments were manufactured thousands of miles away in Fremont, California.

Although not the end of the story, this shipment was a significant milestone in a massive undertaking that had begun in January 2008. In less than a year, the entire manufacturing operation for the Thermo Scientific Autostainer and PT Module has been moved from Fremont to Runcorn in the north west of England. We have made these changes to streamline our instrument manufacturing and development with the entire Thermo Scientific Anatomical Pathology instrument product line.

The Runcorn facility has a long and respected history as a manufacturing site of pathology equipment and has ISO9001:2000 accreditation. Recently, this facility has been upgraded to ISO13485:2003, a quality management standard specifically for medical device manufacturing.

The project team, set up in January 2008, was headed by Allan Lythgoe, Project Manager. Allan has ideal qualifications for managing the integration of the move and had extensive experience having worked at the Runcorn site for 28 years as Operations Manager for the business. The rest of the team consisted of existing Runcorn staff – who had the required skill set in each of their disciplines Quality, Mechanical Engineering, Approvals, Electronics, Software and Sourcing.

The co-operation between the two sites has been exceptional and this has been one of the major highlights of the overall operation and success. None of this would have been possible without the positive attitude and dedication of both Fremont employees and the Runcorn team. Thanks go to everyone involved in making the transition successful!

One of the main challenges we had was to maintain consistency in the supply of instruments and spare parts to our customers during the transition. This was a considerable

undertaking for our suppliers who, at one stage, had to maintain supplies for both sites. We were able to manage this challenge by working intimately with our suppliers. Our thanks go to every supplier who helped us through this process. We will continue to grow the business with this healthy relationship with each supplier. The majority of these suppliers will remain USA based.

Looking to the future, the Runcorn operation is due to move into a larger facility in 2009, which will enable the company to expand and further improve the manufacturing capabilities for the IHC and Anatomical Pathology Instruments as a whole.

New Features

The Runcorn transition provided an ideal opportunity to rationalize and improve the instrument features to better serve customer's needs.

- A **new label sensor** has been introduced to speed up the time taken to scan the vials and the slides, and to increase productivity in the laboratory. For example, on a half full instrument the scan time has been reduced by approximately half.
- All new instruments will have **2D barcode readers** as a standard, allowing for identification of both slides and reagents on each instrument.
- Each of the latest Autostainer options is now supplied with new security fittings for buffer and water inlet tubes and has undergone a number of minor engineering improvements to further enhance performance reliability.

Support

Several new manufacturing team members have been hired in Runcorn and, as with the introduction of any new product, a post launch support team has now been established. This team will meet regularly from January 2009 and will continue to support both the manufacturing process and any field issues concerning the Autostainer and PT Module. You have full support from our International and US teams with our complete portfolio of IHC products.

Autostainer 360-2D

Ordering Information

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A80400002	Thermo Scientific PT Module 110V
A80500004	Thermo Scientific Autostainer 360-2D 110V
A80500010	Thermo Scientific Autostainer 720-2D 110V
A80500024	Thermo Scientific Autostainer 360-2D + PT Module 110V
A80500030	Thermo Scientific Autostainer 720-2D + 2 x PT Module 110V
A80500031	Thermo Scientific Autostainer 720-2D + 1 x PT Module 110V

International

A80400001	Thermo Scientific PT Module 240V
A80500003	Thermo Scientific Autostainer 360-2D 240V
A80500007	Thermo Scientific Autostainer 480S-2D 240V
A80500009	Thermo Scientific Autostainer 720-2D 240V
A80500023	Thermo Scientific Autostainer 360-2D + PT Module 240V
A80500027	Thermo Scientific Autostainer 480S-2D + PT Module 240V
A80500029	Thermo Scientific Autostainer 720-2D + 2 x PT Module 240V

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Pathology Corner:

The Value of IHC in the Realm of Infectious Disease Diagnosis

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Ordinarily, microbial identification of infectious organisms has been made primarily by using serologic assays and cultures[1]. However, both of these methods have their own shortcomings in less than ideal situations; such as when the patient is immunosuppressed or fresh tissue is not available for culture. In addition, culture of fastidious pathogens can be difficult and may take weeks or months to yield results. Even though some microorganisms or their cytopathic effects have distinctive morphologic characteristics that allow their identification in formalin-fixed tissues using routine and special stains, often these changes are not specific or are sparse in the sample evaluated. In these cases, immunohistochemistry will be very valuable to pathologists to render correct diagnosis rapidly so that the patients' treatment can begin.

Immunohistochemistry continues to be one of the most powerful techniques in surgical pathology[2] and there has been an increasing interest in the use of specific antibodies to viral, bacterial, fungal, and parasitic antigens in the detection and identification of the causative agents in many infectious diseases.

Immunohistochemistry plays an important role not only in the diagnosis of numerous viral infections, but also in the study of their pathogenesis and epidemiology. IHC is sufficiently sensitive and specific to differentiate adenovirus colitis from CMV colitis and that of HSV from VZV using monoclonal antibodies. Other salient viral infections in which IHC has been shown to be diagnostically useful include EBV, HHV-8, BK virus, HPV, West Nile virus, parvovirus B19, HBV, HCV, Rabies and viral hemorrhagic fevers such as Ebola virus, yellow fever, Dengue hemorrhagic fever as well as respiratory viral diseases; influenza A virus and respiratory syncytial virus.

Bacterial infections that can be diagnosed by IHC in FFPE tissue include; H. pylori, Whipple disease, Rocky Mountain Spotted Fever, Bartonella infections, Q fever and the spirochetal diseases (leptospirosis, syphilis and most recently Lyme disease[3]).

Several monoclonal and polyclonal antibodies are available to detect fungal elements in formalin fixed tissues when rapid and specific identification is needed to distinguish true infection from harmless colonization. They include antibodies against Candida albicans, C. neoformans, Aspergillus species, Pneumocytis jiroveci (P. carinii), Penicillum marneffei, Sporothrix schenkii, Blastomyces, Coccidioides and Histoplasma. It is important to emphasize that in the case of bacterial and fungal infections, one should be wary of the frequent occurrence of common antigens among bacteria and fungi. Both monoclonal and polyclonal antibodies must be tested for possible cross-reactivities with other organisms before use for diagnosis.

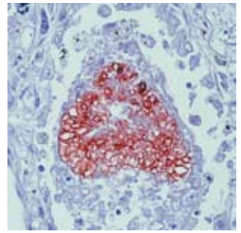
The role of IHC in the identification of protozoal infections is limited to more problematic cases in which the parasite is; few in numbers, present in unusual locations or the morphology is distorted by necrosis. IHC is useful to identify different species of Leishmania, Toxoplasma, Trypanosoma cruzi, Cryptosporidium, Entamoeba histolytica, babesia and Giardia lamblia in FFPE tissue samples.

In addition, IHC has been a very valuable tool in the identification of potential biological terrorism agents (anthrax, plague, tularemia and others) as well as emerging infectious diseases such as hantavirus cardiopulmonary syndrome, corona virus associated with severe acute respiratory syndrome (SARS), and others.

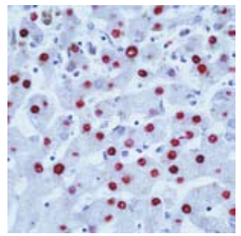
In conclusion, IHC provides a simple, safe, sensitive and specific method for the rapid detection of large number of infectious diseases and is especially useful when microorganisms are difficult to identify by routine or special stains, are fastidious to grow, or exhibit an atypical or very similar morphology.

Reference the chart on the right for more Thermo Scientific IHC markers used for diagnosis of infectious diseases.

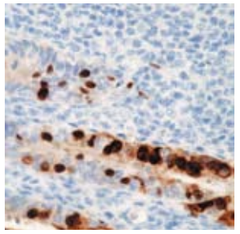
ANTIBODY NAME	CLONE	CAT #
Adenovirus	M58+M73	MS-1069
Adenovirus Type2 E1AAb2	M73	MS-588
Cytomegalovirus	CMV01	MS-717
Epstein-Barr Virus / LMP	CS1+CS2+CS3+CS4	MS-1458
Helicobacter pylori	Poly	RB-9070
Hepatitis B Virus Core Antigen (HBVcAg)	Poly	RB-1413
Hepatitis B Virus Surface Antigen (HBVsAg)	T9; Same as 3E7	MS-314
Herpes Simplex Virus Type 2 (HSV-II)	Poly	RB-1426
Human Papilloma Virus (HPV)	K1H8	MS-1826
Pneumocystis jiroveci (P. carinii)	3F6	MS-1427
Toxoplasma gondii	Poly	RB-282



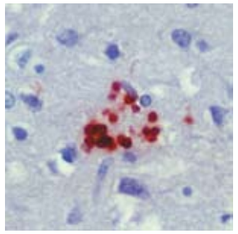
Pneumocystis jiroveci (P. Carinii)
Clone: 3F6 **Cat #:** MS-1427



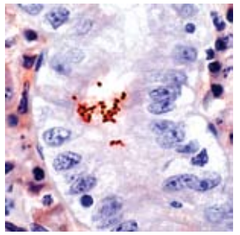
Hepatitis B Virus Core Antigen (HBVcAg)
Clone: Poly **Cat #:** RB-1413



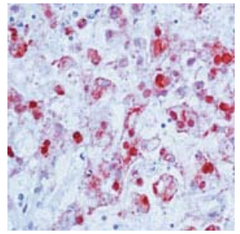
Adenovirus Type 2 E1AAb2
Clone: M73 **Cat #:** MS-588



Toxoplasma gondii
Clone: Poly **Cat #:** RB-282



Helicobacter pylori
Clone: Poly **Cat #:** RB-9070



Herpes Simplex Virus Type 2
Clone: Poly **Cat #:** RB-1426

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References:
1. E. Eyzaguirre; A. K. Haque. Arch Pathol Lab Med. 2008.132
2. D. J. Dabbs. Diagnostic Immunohistochemistry. PP. 43-64
3. Eisendle K. et al. Amer J Clin Pathol. 2007. 127; 213:222.

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Florence, Italy
Exhibit dates: September 4-9, 2009

National Society for Histotechnology (NSH)
Birmingham, Alabama
Exhibit dates: October 3-8, 2009

College of American Pathologists (CAP)
San Diego, California
Exhibit dates: October 11-14, 2009

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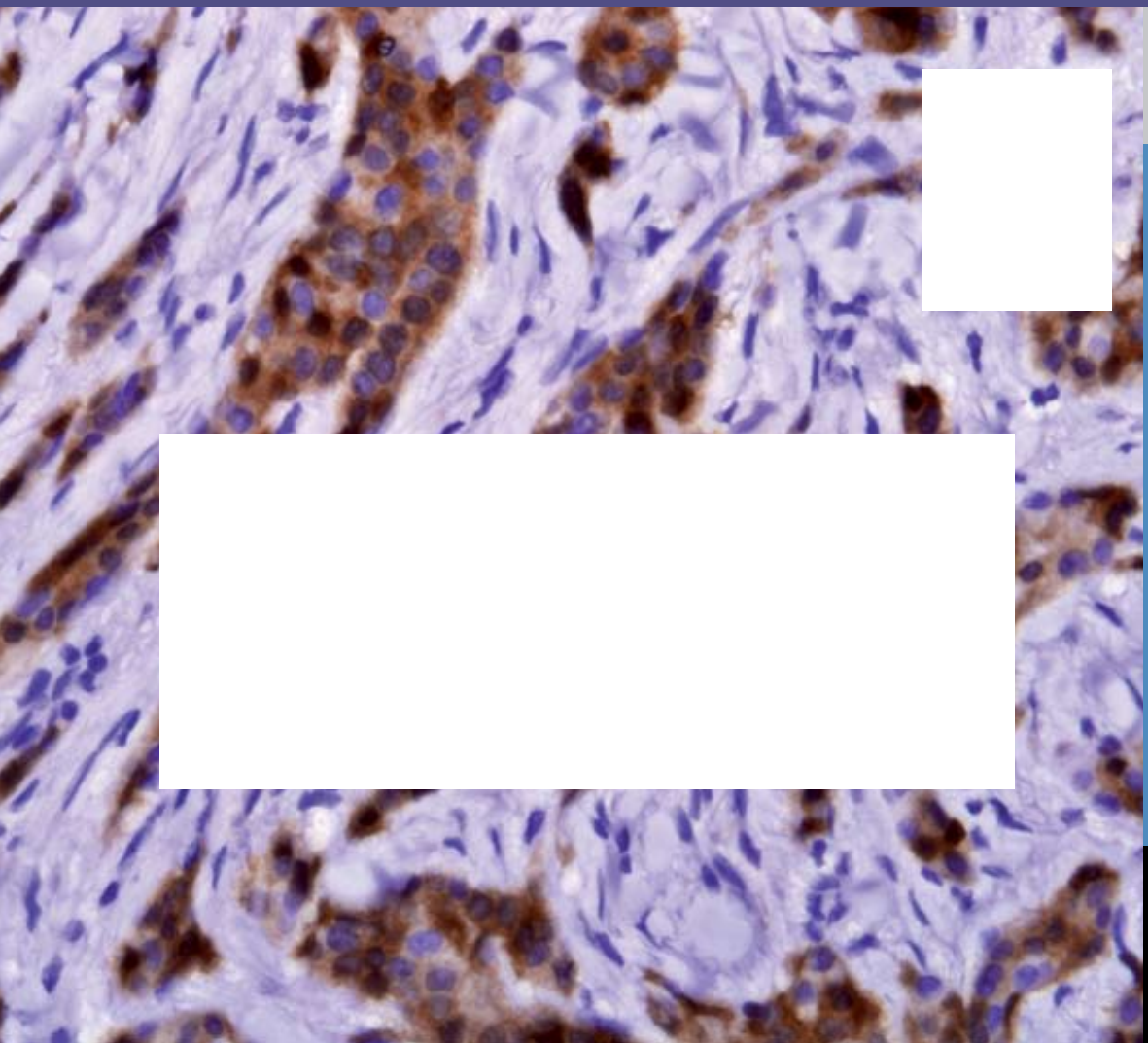

Breast Carcinoma Stained with NY-BR-1 (MS-1932)

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