NEW

ZETA Corporation

Precision IVD Antibodies for Anatomic Pathology

NEW PRODUCT FOCUS -- Zeta Dual Stain Antibody Cocktails

ZETA introduces its new line of dual stain antibody cocktails, specifically designed to deliver more information than traditional single color, single antibody products.

Our pathologists carefully curate useful antibody combinations and fine-tune their performance in the lab to work together with accuracy and precision. The use of two-color staining allows review of the specific expression identifiable to each antibody, providing a significant increase in information from one slide. Each dual stain cocktail provides the high quality that you have come to expect from Zeta combined with meaningful, diagnostically relevant information. All products come in our easy, Ready-To-Use format.

Zeta Corporation is a leader in high quality clinical recombinant antibodies developed specifically for the anatomic pathology market for Immunohistochemistry. Developed by a pathologist for pathologists, Zeta target-validates and characterizes each primary antibody for IHC on FFPE tissue sections. Zeta provides 400+ IVD antibodies for cancer screening and diagnosis. Zeta maintains the highest quality for IVD products and is certified ISO13485/ cGMP/IVDR/MDSAP, manufacturing all products in the USA through recombinant production. When reviewing IHC results on patient tumor tissue samples, the pathologist evaluates many factors, cellular morphology and sometimes many markers.

Bone Marrow Cocktail

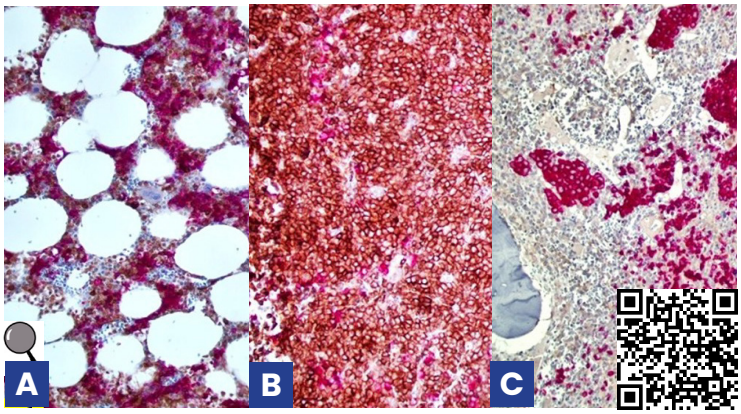
(CD71 + CD33)

IVD US, non EU; EU: RUO

The combination of anti-CD33 (brown) and anti-CD71 (red) stain is useful to identify most blasts (myeloid and erythroid blasts) in bone marrow.

A cocktail of monoclonal antibodies for IHC including CD71 and CD33. CD33 is a glycosylated transmembrane protein. Acute myeloid leukemia, acute monocytic leukemia (AML-M5), and all cases of myeloid sarcoma are positive for CD33. The excellent sensitivity and specificity for myelomonocytic lineage makes **CD33 (mouse clone PSW44)** a useful diagnostic marker for myeloid sarcoma. **CD71 (rabbit clone ZR422)** is highly specific, and recognizes cell surface transferrin receptor (CD71). This receptor has been observed expressed by normal erythroid and erythroid leukemias. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2841



IHC: normal human bone marrow **(A)**, myeloid leukemia **(B)**, and bone marrow erythroid hyperplasia **(C)** stained with Bone Marrow Cocktail antibodies using DAB-conjugate anti-mouse (CD33) and AP-conjugated anti-rabbit (CD71). Note the myeloid cells are positive for CD33 (brown) and erythroid cells are positive for CD71 (red).

Breast Cocktail

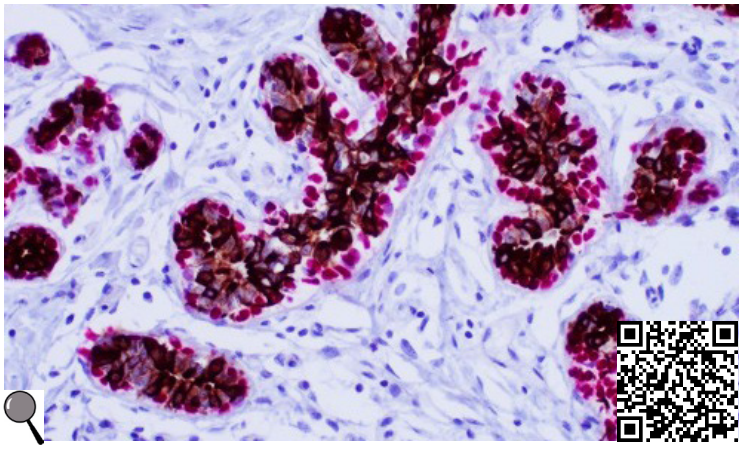
(p63 + CK8/18)

IVD US, non EU; EU: RUO

The combination of p63 (red) and CK8/18 (brown) immunohistochemical stain is used to identify breast invasive ductal and lobular carcinoma (CK8/18 positive, P63 negative) against normal breast tissue or in situ breast carcinoma (CK8/18 positive, P63 positive)

p63 is a homolog of the tumor suppressor p53 identified in basal cells in the epithelial layers of a variety of tissues including breast and prostate. Cytokeratin 8 (CK8) belongs to the type II subfamily of high MW CKs and exists in combination with CK18. **p63 (rabbit clone ZR439)** was detected in nuclei of the basal epithelium in normal breast tissue. Cytokeratin **CK8/18 (mouse clone B22.1/B23.1)** recognizes all simple epithelia including glandular epithelium i.e. thyroid, breast, gastrointestinal tract etc. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2833



IHC: Human normal breast stained with Breast Cocktail Antibodies using DAB-conjugate anti-mouse (CK8/18) and AP-conjugated anti-rabbit (p63). Note the luminal cells are positive for CK8/18 (brown) and myoepithelial cells are positive for P63 (red)

New detection kits, stains and related reagents >>> pages 5-6

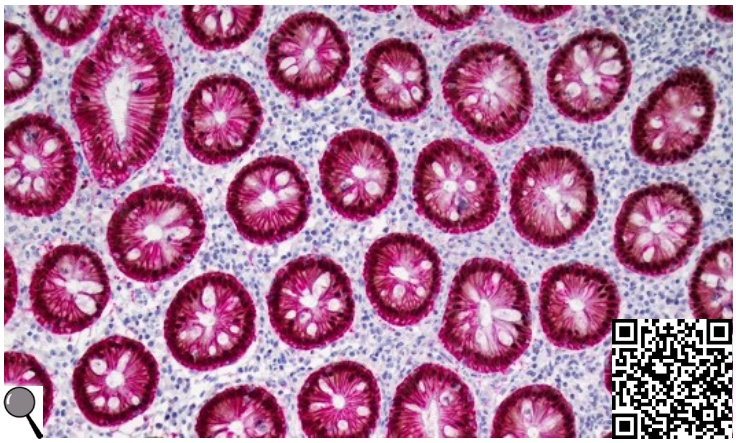
Colon Cocktail
(Cadherin 17 + CDX-2)

IVD US, non EU; EU: RUO

The combination AP-labeled cadherin 17 (red) and HRP-labeled CDX-2 (brown) paraffin immunohistochemistry is highly sensitive and can indicate colorectal and stomach adenocarcinoma.

Zeta's recombinant rabbit antibodies recognize **Cadherin 17 (rabbit clone ZR418)** and **CDX-2 (mouse clone ZM20)**, are novel diagnostic markers for GI adenocarcinoma. Cadherin 17 (CDH17) is involved in tumor invasion and metastasis and a highly specific marker in colon cancer. CDX-2 is a homeobox gene that encodes an intestine-specific transcription factor. Anti-CDX-2 has been useful to establish GI origin of metastatic adenocarcinomas and carcinoids and is useful to distinguish metastatic colorectal adenocarcinoma from lung adenocarcinoma. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2840



IHC: Human colon stained with Colon Cocktail Antibodies using DAB-conjugate anti-mouse (CDX-2) and AP-conjugated anti-rabbit (cadherin 17). The crypt cells are nuclear positive for CDX-2 (brown) and cytoplasmic positive for cadherin 17 (red)

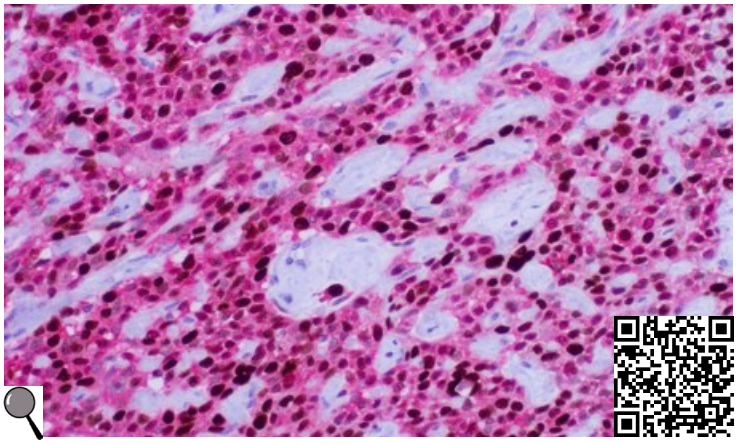
HPV-Infected Tumor Cocktail
(p16^{INK4} + Ki-67)

IVD US, non EU; EU: RUO

A cocktail to identify tumor cells co-expressing both tumor suppression marker P16 (red) and cell proliferation marker Ki-67 (brown) in HPV infected tumors, such as high-grade dysplasia and carcinomas.

This cocktail contains **p16^{INK4a} (rabbit clone ZR407)** and **Ki-67 (mouse clone ZM67)** that both recognize HPV. p16^{INK4a} is a tumor suppressor protein involved in the pathogenesis of a variety of malignancies. Tumors infected with HPV, particularly the high-risk stains, express high level of P16 protein. The Ki-67 nuclear antigen is associated with cell proliferation. It is found throughout the cell cycle in the G1, S, G2, and M phases; but not the (G0) phase, and used to grade proliferation rates of tumors. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2835



IHC: Human cervical HPV-associated squamous cell carcinoma (SCC) stained with HPV Infection Cocktail Antibodies using DAB-conjugate anti-mouse (Ki-67) and AP-conjugated anti-rabbit (P16). Note the SCC is strongly positive for P16 (red) with a high Ki-67 index (~50%) (brown)

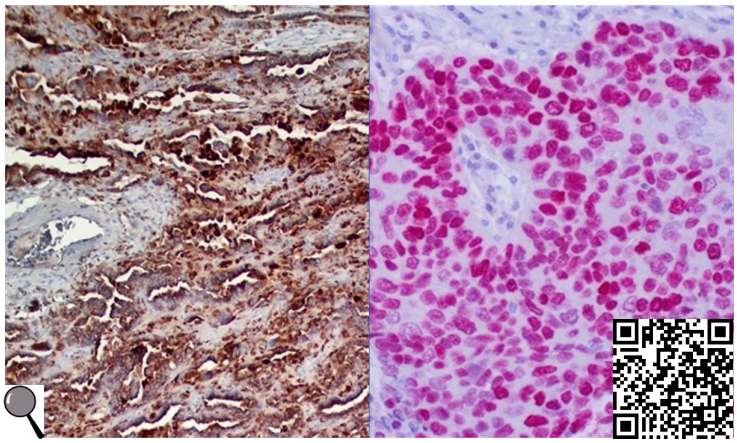
Lung Cocktail
(P40 + Napsin A)

IVD US, non EU; EU: RUO

The high specificity expression of Napsin A (brown) in lung adenocarcinomas is useful to distinguish primary lung adenocarcinomas from lung squamous cell carcinoma (P40 positive, red) and adenocarcinomas of other organs.

P40 (rabbit clone ZR8) and **Napsin A (mouse clone ZM11)**, are novel diagnostic markers for adenocarcinoma of the digestive system. p63 consists of two major isoforms-TAp63 and DNp63. DNp63 isoform (identified by anti-p40) contains an alternative transcriptionally inactive domain. ZR8 recognizes DNp63 but not TAp63. p40 reacts with squamous cell carcinomas but not with adenocarcinomas. Napsin is a pepsin-like aspartic proteinase consisting of Napsin A and Napsin B. Napsin A is expressed in type II pneumocytes and in lung adenocarcinomas. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2839



IHC: Human lung adenocarcinoma (left) and lung squamous cell carcinoma (right) stained Lung Cocktail antibodies with DAB-conjugate anti-mouse (napsin A) and AP-conjugated anti-rabbit (P40). Note the adenocarcinoma is only positive for Napsin A (brown) whereas the squamous cell carcinoma is only positive for p40 (red).

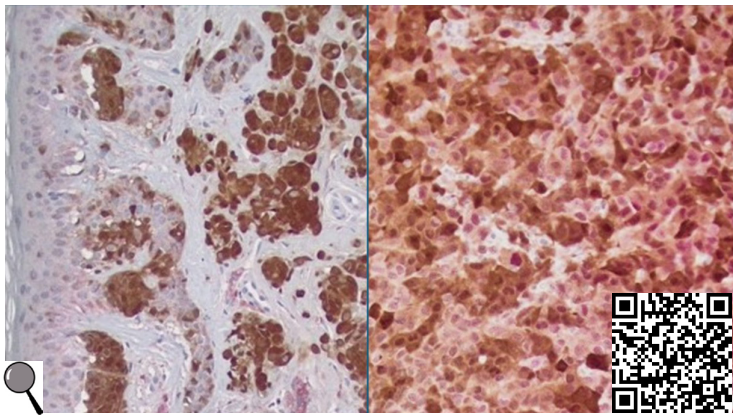
Melanoma Cocktail
(PRAME + S-100)

IVD US, non EU; EU: RUO

The combination of PRAME (red) and S-100 (brown) IHC staining can aid in differentiating malignant melanoma and atypical nevus (PRAME positive, S-100 positive) from benign nevus (PRAME negative, S-100 positive).

PRAME is a transcriptional repressor that prevents retinoic acid-induced cell proliferation arrest, differentiation, and apoptosis. This gene encodes an antigen (used to produce Zeta anti-PRAME, **rabbit clone ZR383**) that is preferentially expressed in human melanomas and is recognized by cytolytic T lymphocytes. Not expressed in normal tissues, except testis. S-100 is a calcium binding protein expressed in melanocytes and the antigen presenting cells. S-100 **(mouse clone 4C4.9)** stains Schwannomas, ependymomas, astrogliomas, almost all benign and malignant melanomas and their metastases. ***(MORE)***

Species: rabbit & mouse monoclonals **Cat#:** Z2838



IHC: Human compund nevus (left) and malignant melanoma (right) stained with Melanoma Cocktail using DAB-conjugate anti-mouse (S-100) and AP-conjugated anti-rabbit (PRAME). Note the nevus cells are only positive for S-100 (brown) and negative for PRAME, whereas melanoma cells are positive for both PRAME (nuclear, red) and S-100 (nuclear and cytoplasmic, brown)

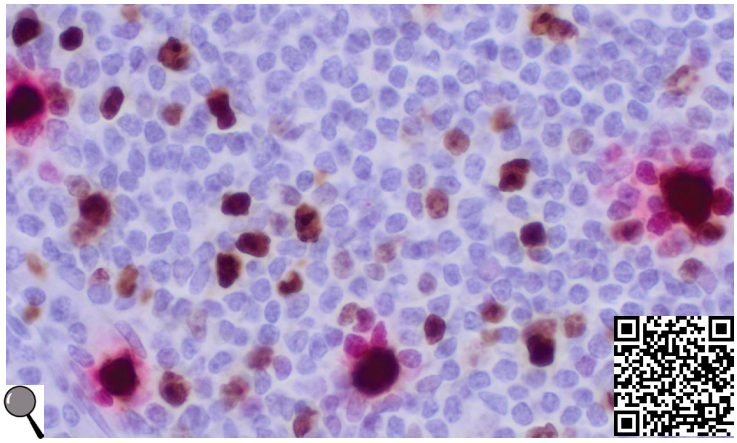
Proliferation Cocktail
(PHH3 + Ki-67)

IVD US, non EU; EU: RUO

The combination of PHH3 (red) and Ki-67 (brown) immunohistochemical staining can help evaluate tumor grade (neuroendocrine tumor), and malignancy (uterine smooth muscle tumors, CNS tumors, GIST, melanocytic tumors).

Phosphohistone H3 (PHH3) is a mitotic marker. Serine 10 of Histone H3 is phosphorylated in association with mitotic chromatin condensation in late G2 and M phase of the cell cycle and thus, PHH3 **(rabbit clone ZR285)** can distinguish mitosis from apoptotic nuclei. High level of PHH3 is associated with increased mitotic count and with high Ki-67 expression. Ki-67 antigen is a nuclear, non-histone protein that is present in all stages of the cell cycle except G0. This characteristic makes Ki-67 **(mouse clone ZM67)** an excellent marker for proliferating cells. ***(MORE)***

Species: rabbit & mouse monoclonals **Cat#:** Z2834



IHC: Human tonsil stained with Proliferation Cocktail Antibodies using DAB-conjugate anti-mouse (Ki-67) and AP-conjugated anti-rabbit (PHH3). Note the four mitotic cells are positive for both Ki-67 (brown) and PHH3 (red) and proliferative cells are positive for Ki-67 (brown).

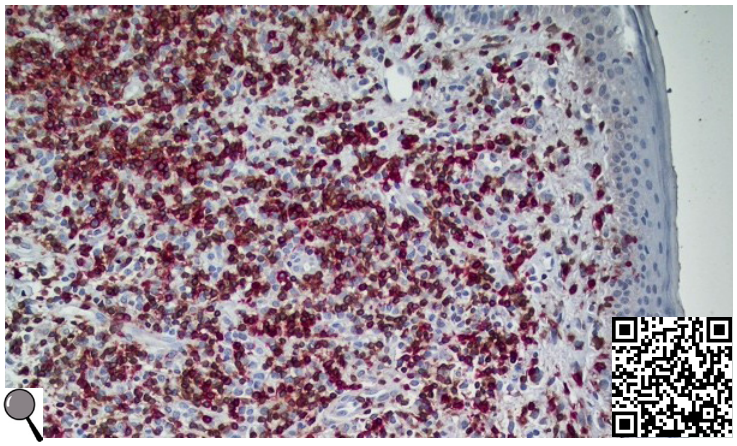
T Cell Clonality Cocktail
(TRBC1 + CD3)

IVD US, non EU; EU: RUO

The combination of CD3 and TRBC1 antibodies can be used to determine T cell clonality by paraffin immunohistochemistry. The staining CD3/TRBC1 patterns: normal lymphoid tissue: mixed CD3 (brown) and TRBC1 (red), ratio 2:1; monoclonal T cell population: mixed CD3 (brown) and TRBC1 (red), ratio 1:1, or only brown with no red staining.

Antibodies to **CD3** (**mouse clone ZM45**) and **TRBC1** (**rabbit clone ZR481**) stain human T cells in both the cortex and medulla of the thymus and in peripheral lymphoid tissues and is suitable for staining normal and neoplastic T cells. TRBC1 refers to T-Cell Receptor Beta Constant 1;. Malignant lymphomas and leukemias often show restricted expression of TRBC1 or TRBC2, rather than normal mixed making CD3 and TRBC1 antibodies useful in clonality assessment. ***(MORE)***

Species: rabbit & mouse monoclonals **Cat#:** Z2854



IHC: Human mycosis fungoides stained with T Cell Clonality cocktail antibodies using DAB-conjugate anti-mouse (CD3) and AP-conjugated anti-rabbit (TRBC1). Note the monoclonal T cells are positive for both CD3 (brown) and TRBC1 (red).

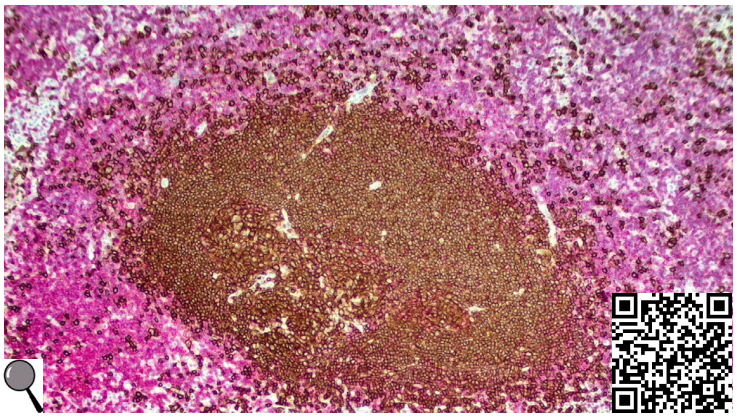
T/B Cell Cocktail
(CD3 + CD20)

IVD US, non EU; EU: RUO

The combination of CD3 (red) and CD20 (brown) antibodies allow B-cell and T-cell lymphocytes to be identified on the same slide.

Anti-**CD3 (rabbit clone ZR414)** recognizes the epsilon chain of CD3, which consists of five different polypeptide chains (designated as gamma, delta, epsilon, zeta, and eta). The CD3 antigen is a highly specific marker for T cells and has been reported expressed by most T cell neoplasms. CD20 is a non-Ig differentiation antigen of B cells and the expression of CD20 is restricted to normal and neoplastic B cells, being absent from all other leukocytes and tissues. **CD20 (mouse clone L26)** is the most specific B-cell marker used in paraffin immunohistochemistry. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2855



IHC: Human lymph node stained with T/B Cell Cocktail Antibodies using DAB-conjugate anti-mouse (CD20) and AP-conjugated anti-rabbit (CD3). Note the folliclar B cells are positive for CD20 (brown) and perifollicular T cells are positive for CD3 (red).

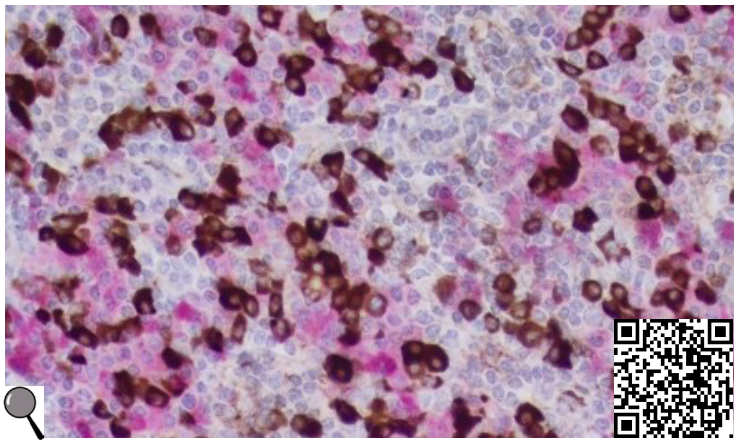
B Cell Clonality Cocktail
(Kappa + Lambda)

IVD US, non EU; EU: RUO

The cocktail can help establish B cell/plasma cell clonality where AP-labeled kappa (red) and HRP-labeled lambda antibodies (brown) are visualized through paraffin immunohistochemistry on a single slide.

B cells are a type of white blood cell that produce antibodies. Clonality refers to the process where a single B cell undergoes division to produce a population of identical cells which secrete monoclonal Ig with either kappa or lambda light chains. Antibodies are used to determine the ratio of **kappa (rabbit clone ZR472)** to **lambda (mouse clone LcN2)** light chains. In normal lymphoid tissue, the kappa and lambda cell ratio are approximately 0.3-1.7. In the hematological diseases, such as B-cell lymphomas or B-cell leukemias, there is presence of abnormal... *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2837



IHC: Human lymph node stained with B Cell Clonality Cocktail using DAB-conjugate anti-mouse (I ambda) and AP-conjugated anti-rabbit (kappa). Note the polyclonal plasma cells are positive for kappa light chain (red) and lambda light chain (brown)

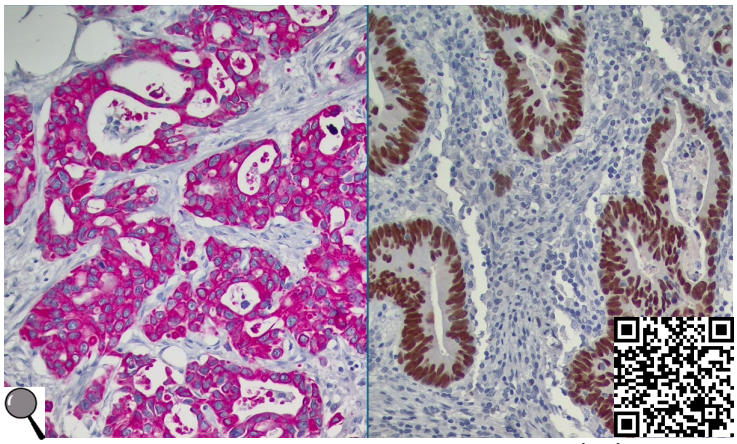
Unknown Primary Cocktail (UPC)
(CK7 + CDX-2)

IVD US, non EU; EU: RUO

The combination of CK7 (red) and CDX-2 (brown) antibodies can separate unknown primary carcinomas (UNC) as CK7+/CDX-2- (breast, GYN, lung) CK7+/CDX-2+ (bladder, stomach, appendix, ovarian mucinous carcinoma), CK7-/CDX-2+ (colorectal), or CK7-/CDX-2- (prostate, kidney).

Cytokeratin 7 is found in glandular and transitional epithelia but not in the stratified squamous epithelia. It's expressed in epithelial cells of ovary, lung, and breast but not of colon, prostate, or GI tract. Anti-CK7 **(rabbit clone ZR428)** can distinguish ovarian carcinomas (CK7+) from colon carcinomas (CK7-). CDX-2 is expressed in GI organs and GI carcinomas. Anti-CDX-2 **(mouse clone ZM20)** can establish GI origin of metastatic adenocarcinomas and carcinoids and distinguishes metastatic colorectal adenocarcinoma from lung adenocarcinoma. *(MORE)*

Species: rabbit & mouse monoclonals **Cat#:** Z2856



IHC: Human metastatic pancreatic ductal carcinoma (left) and metastatic colon adenocarcinoma (right) stained with Unknown Primary Cocktail Antibodies using DAB-conjugate anti-mouse (CDX-2) and AP-conjugated anti-rabbit (CK7). Note the pancreatic ductal carcinoma is CK7+/CDX-2, whereas the colon adenocarcinoma is CK7+/CDX-2-.

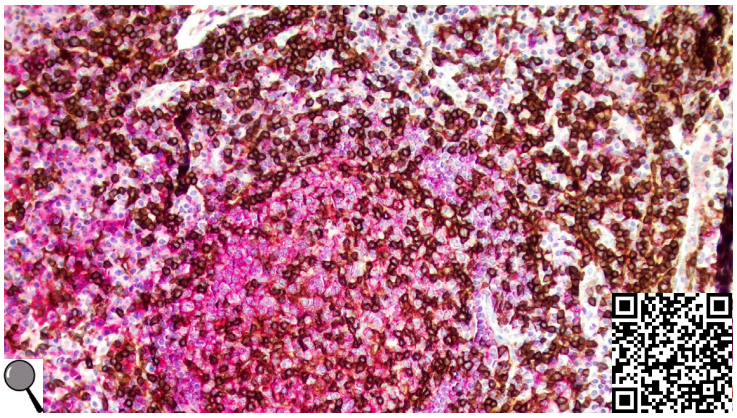
T Cell Cocktail
(CD4 + CD8)

IVD US, non EU; EU: RUO

The combination of CD4 (brown) and CD8 (red) is helpful in distinguishing mycosis fungoides (CD4+). Multiplex IHC may also give distinct advantages if ratios and/or cell counts on a single slide are desired.

Zeta's T-CELL COCKTAIL is a DUAL STAIN product that recognized both CD4 **(mouse clone ZM180)** and CD8 **(rabbit clone ZR286)** to help identify T-cell subpopulations and provides visualization of the immune cell composition within the tissue microenvironment by enabling two chromogen detection on one slide. In cancer prognosis, the T-Cell Cocktail can provide predictive immune response information; in autoimmune and inflammation, it can characterize the infiltrate type (CD4 versus CD8 dominant); and in transplant monitoring, it can detect rejection or graft-versus-host disease (GVHD). CD4 is expressed in a T-cell subset (helper/inducer) and is ... ***(MORE)***

Species: rabbit & mouse monoclonals **Cat#:** Z2823



IHC: Human tonsil stained with T Cell Cocktail Antibodies using DAB-conjugate anti-mouse CD4 (clone ZM180) and AP-conjugated anti-rabbit CD8 (clone ZR286). Note the perifollicular T cells are either positive for CD4 (brown) or CD8 (red).

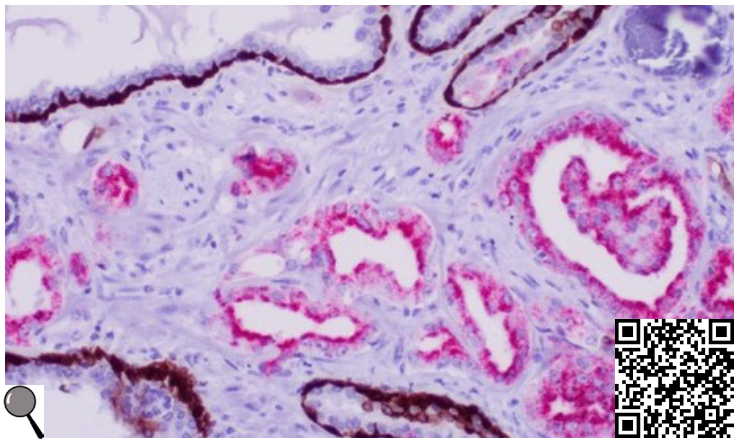
Prostate Cocktail
(AMACR + p63 + CK-HMW)

IVD US, non EU; EU: RUO

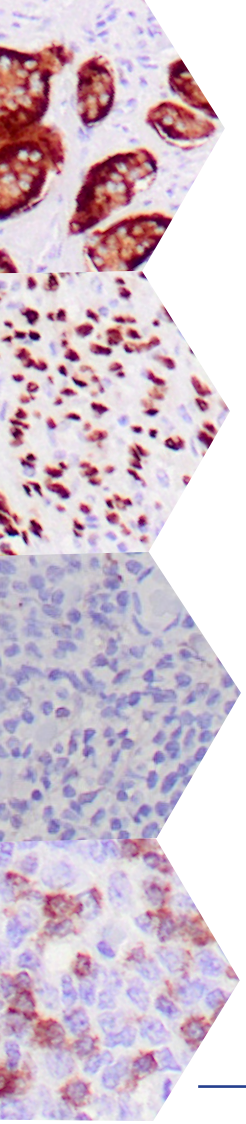
The combination of AMACR (brown) and p63 (red) may be extremely useful for diagnosing prostate intraepithelial neoplasia (PIN) and small focus adenocarcinoma, especially in difficult cases and cases with limited tissues. AMACR stains cytoplasm in prostate adenocarcinoma and PIN while p63 stains basal cell nuclei in PIN and benign prostate gland.

A cocktail of monoclonal antibodies for IHC including AMACR **(rabbit clone 13H4)**, p63 **(mouse clone 4A4)**, and CK-HMW **(mouse clone 34βE12)**. AMACR is an essential enzyme in the β-oxidation of branched-chain fatty acids. High expression of AMACR protein is found in prostate adenocarcinoma but not in benign prostate tissue by immunohistochemical staining in paraffin-embedded tissue. The expression of AMACR is also detected in prostate premalignant lesions, such as prostate intraepithelial neoplasia (PIN). The p63 protein, a... ***(MORE)***

Species: rabbit & mouse monoclonals **Cat#:** Z2836



IHC: Human prostate adenocarcinoma stained with Prostate Cocktail Antibodies using DAB-conjugate anti-mouse (HMW CK & p63) and AP-conjugated anti-rabbit (AMACR). Note the benign glands (top and bottom) are p63+/HMW CK34βE12+/AMACR- whereas adenocarcinoma (center) is p63-/HMW CK-34βE12-/AMACR+.



Detection Kits and Reagents
for FFPE Immunocytochemistry

Zeta Corporation offers polymer-based detection to reduce non-specific staining and increase the signal-to-noise ratio of the immunohistochemistry application (IHC). Many of the human organs contain endogenous biotin that might interfere with the actual staining for IHC and require additional blocking steps. With the advent of polymer detection technology, the biotin was completely removed from the detection chemistry and thus reducing the "background" noise. However, the size of the polymer molecule also introduced the steric hindrance. Therefore, Zeta made sure that our detection polymers are small in size to avoid steric hindrance and help increase the sensitivity and specificity of the detection process.

We've developed two different detection chemistries to fit every laboratory need. They are both universal detection chemistries, meaning they can detect both mouse and rabbit primary antibodies on human tissue specimens. To offer more sensitive detection for some of the low-expressing nuclear primary antibodies, Zeta introduced the Zeta MAX detection kits with an amplification step specifically targeting mouse primary antibodies.

Zeta Universal HRP/AP Polymer Detection

A primary antibody specific to an antigen on a formalin-fixed paraffin-embedded (FFPE) tissue section is detected by the Zeta Universal HRP Polymer Detection Kit. The antigen sites are then visualized with DAB chromogen/substrate. This kit is a one-step system using a direct method resulting in a polymer-secondary antibodies-HRP complex that universally detects mouse and rabbit primary antibodies. The resulting chromogenic reaction can be visualized by HRP-compatible chromogens using light microscopy.

Polymer-based detection reduces non-specific staining and increases the signal-to-noise ratio of immunohistochemistry. Many human organs contain endogenous biotin that might interfere with the actual staining for IHC and require additional blocking steps. With polymer detection technology, biotin is completely removed from the detection chemistry thereby reducing the "background" noise. Zeta ensured that our detection polymers are small in size to avoid steric hindrance and help increase the sensitivity and specificity of the detection process.

Zeta Max

The Zeta Max HRP Polymer Detection Kit uses an amplifying reagent (Zeta Max Amplifier) in conjunction with Zeta HRP Polymer (anti-mouse HRP/anti-rabbit HRP) to increase the signal intensity of primary antibodies. The mouse primary antibody specific to an antigen on the formalin-fixed paraffin-embedded (FFPE) tissue section is detected by the Zeta Max Amplifier. The Amplifier reagent is then detected by Zeta HRP Polymer. The Zeta HRP Polymer and the Zeta Max Amplifier reagents are ready-to-use and provided in convenient dropper bottles.

Zeta MAX utilizes unique technology to amplify mouse primary antibodies and particularly antibodies expressed in the nucleus. Zeta MAX reagent penetrates the tissues to tag the primary antibody sites to amplify and generate a multitude of sites for the micro-HRP polymer to bind.

Product details and ordering information, page 7 →

Zeta Universal Polymer Detection

Zeta Universal HRP Polymer Detection Kit (with DAB) **Cat#: ZD11**

Reagents: Anti-mouse HRP/anti-rabbit HRP (100 ml)
DAB Chromogen Concentrate (6.25 ml)
DAB Substrate Buffer (118.75 ml)

Zeta Universal HRP Polymer Detection (without DAB) **Cat#: ZD10**

Reagent: Anti-mouse HRP/anti-rabbit HRP (100 ml)

NEW

Zeta Universal AP Polymer Detection Kit **Cat#: ZD12**

Reagents: Anti-mouse AP/anti-rabbit HRP (100 ml)
AP Red Chromogen

NEW **Zeta AP Red Chromogen** (with buffer) **Cat#: ZD16**

Zeta MAX Polymer Detection

Zeta MAX Kit (with DAB) **Cat#: ZD15**

Reagents: Zeta HRP Polymer (anti-mouse HRP/ anti-rabbit HRP) (ready-to-use) (100 ml)
Zeta Max Amplifier reagent (100 ml)
DAB Chromogen Concentrate (6.25 ml)
DAB Substrate Buffer (118.75 ml)

Zeta MAX Kit (without DAB) **Cat#: ZD14**

Reagents: All of the above minus DAB dhromogen and substrate buffer

NEW

Zeta Dual AP/HRP Detection Kit **Cat#: ZD9**

The primary antibody specific to an antigen on formalin-fixed paraffin-embedded (FFPE) tissue section is detected by the Zeta Dual AP/HRP Polymer Detection Kit. The antigen sites are then visualized with AP/HRP chromogen/substrate. The reagent is ready-to-use in a convenient dropper bottle. The Zeta Universal HRP Polymer Detection Kit is a one-step system that uses a direct method, resulting in a polymer-secondary antibodies anti-Rabbit AP /anti-mouse HRP complex that universally detects primary mouse and rabbit antibodies. The resulting chromogenic reaction can be visualized by AP/HRP-compatible chromogens using light microscopy. The staining signals can be amplified with enhancers if necessary.

Detection Reagents and Tools

NEW **Zeta MAX Antibody Diluent** **Cat#: ZD20**
Ready-to-use (Tris Buffered) diluent for diluting primary antibodies and for use as a negative control.

NEW **Zeta MAX Block** **Cat#: ZD7**
For serum-free blocking of non-specific antibody binding in IHC. More effective at reducing non-specific background staining than serum.

NEW **Zeta Peroxide Block** **Cat#: ZD18**
Developed for use in immunolabeling techniques to reduce non-specific background staining due to endogenous peroxidase.

Zeta Antibody Diluent, 500 ml **Cat#: ZD19**
Antibody Diluent is provided as a ready-to-use (Tris Buffered) diluent for diluting primary antibodies and for use as a negative control.

Zeta Citrate Plus HIER Solution (10X), 1L **Cat#: ZD2**
Citrate Plus (10X) HIER Solution is a unique citrate buffer designed to significantly enhance immunohistochemical staining.

Zeta Tris-EDTA HIER Solution (10X), 500 ml **Cat#: ZD6**
Tris-EDTA HIER Solution (10x) pH 9.0) is a unique buffer designed to significantly enhance immunohistochemical staining.

Zeta TBS plus Tween 20 pH 7.4, (20X) 500 ml **Cat#: ZD5**
Optimal formulation of pH stabilizers, salts and detergents for removal excess material from the tissue sample.

Zeta PBS plus Tween 20 pH 7.6 (20X), 1L **Cat#: ZD3**
Optimal formulation of pH stabilizers, salts and detergents designed to effectively remove excess material from microtiter plate wells.

Zeta PAP Pen **Cat#: ZD1**
PAP-Pen (Liquid Blocker) makes a water repellent barrier for manual IHC staining.