

**CK5/14 + p63 + P504S**  
Prediluted Multiplex Antibody Reagent  
Control Number: 902-225DS-082517

<b>Catalog Number:</b>	<b>PPM 225 DS AA, H</b>	<b>IPR 225DS G10</b>
<b>Description:</b>	6.0, 25 ml, prediluted	10 ml, prediluted
<b>Dilution:</b>	Ready-to-use	Ready-to-use
<b>Diluent:</b>	N/A	N/A

**Intended Use:**

For Research Use Only. Not for use in diagnostic procedures.

**Summary and Explanation:**

CK5 and CK14 are high molecular weight cytokeratins expressed in a variety of normal and neoplastic epithelial tissues (1). In prostate tissue, mRNA for CK5 and CK14 has been detected in the basal cells of normal glands and prostatic intraepithelial neoplasia (PIN), a precursor lesion to prostatic adenocarcinoma; however, expression of CK5 or CK14 was not identified in invasive prostatic adenocarcinoma (2).

p63, a homolog of the tumor suppressor p53, has been identified in proliferating basal cells in the epithelial layers of a variety of tissues, including epidermis, cervix, urothelium and prostate (3). p63 was detected in nuclei of the basal epithelium in normal prostate glands; however, it was not expressed in malignant tumors of the prostate (4).

P504S, also known as  $\alpha$ -methylacyl coenzyme A racemase (AMACR), is a peroxisomal and mitochondrial enzyme that plays a role in bile acid synthesis and  $\beta$ -oxidation of branched chain fatty acids (5). P504S was initially identified from a cDNA library as a gene that is overexpressed in human prostate cancer; with little or no expression in normal prostate (6,7). In immunohistochemistry, P504S has been shown to be a specific marker of prostatic adenocarcinoma (6-9). Additionally, prostate glands involved in PIN have been found to express P504S, whereas P504S was nearly undetectable in benign glands (9,10). U.S. Patent 8,603,765 and patents pending.

**Principle of Procedure:**

This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

**Reagent Provided:**

CK5 + CK14 + p63 + P504S\* is provided as a prediluted antibody cocktail of anti-CK5, anti-CK14, anti-p63, and anti-P504S antibodies, in buffer with carrier protein and preservative. \*Product formerly known as PIN-4®

Antibody	anti-CK5	anti-CK14	anti-p63	anti-P504S
Clone	XM26	LL002	4A4	N/A
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Rabbit polyclonal
Isotype	IgG1/kappa	IgG3	IgG2a/kappa	IgG
Epitope/Antigen	CK5	CK14	p63	P504S
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear	Granular cytoplasm
Staining	Brown (DAB)	Brown (DAB)	Brown (DAB)	Red (Warp Red)

**Storage and Stability:**

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

**Known Applications:**

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Species Reactivity:** Human, others not tested

**Positive Tissue Control:** Normal prostate and prostatic adenocarcinoma

**Staining Protocol Recommendations (intelliPATH and manual use):**

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Biocare's Reveal Decloaker or Diva Decloaker. Refer to the Reveal Decloaker or Diva Decloaker product data sheet for specific instructions.

**Protein Block:** Incubate for 10 minutes at RT with Biocare's Background Punisher.

**Primary Antibody:** Incubate for 30-45 minutes at RT.

**Double Stain Detection:** Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2 or intelliPATH Multiplex Secondary Reagent 2.

**Chromogen (1):** Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

**Chromogen (2):** Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

**Counterstain:** Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

**intelliPATH™ Automated Slide Stainer:**

IPR225DS is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

**Technical Note:**

This antibody has been optimized for use with Biocare's MACH 2 Double Stain 2 and intelliPATH Multiplex Secondary Reagent 2. Use TBS for washing steps.

**Limitations:**

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

**Precautions:**

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (11)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (12)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

**Technical Support:**

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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**References:**

1. Moll R, *et al.* The Catalog of Human Cytokeratins: Patterns of Expression in Normal Epithelia, Tumors and Cultures Cells. *Cell*. 1982 Nov; 31(1):11-24.
2. Yang Y, *et al.* Differential Expression of Cytokeratin mRNA and Protein in Normal Prostate, Prostatic Intraepithelial Neoplasia, and Invasive Carcinoma. *Am J Pathol*. 1997 Feb; 150(2):693-704.
3. Yang A, *et al.* p63, a p53 Homolog at 3q27-29, Encodes Multiple Products with Transactivating, Death-Inducing, and Dominant-Negative Activities. *Mol Cell*. 1998 Sep; 2(3):305-16.
4. Signoretti S, *et al.* p63 Is a Prostate Basal Cell Marker and Is Required for Prostate Development. *Am J Pathol*. 2000 Dec; 157(6):1769-75.
5. Ferdinandusse S, *et al.* Subcellular localization and physiological role of  $\alpha$ -methylacyl-CoA racemase. *J Lipid Res*. 2000 Nov; 41(11):1890-6.
6. Xu J, *et al.* Identification of Differentially Expressed Genes in Human Prostate Cancer Using Subtraction and Microarray. *Cancer Res*. 2000 Mar 15; 60(6):1677-82.
7. Rubin MA, *et al.*  $\alpha$ -Methylacyl Coenzyme A Racemase as a Tissue Biomarker for Prostate Cancer. *JAMA*. 2002 Apr 3;287(03):1662-70.
8. Luo J, *et al.* Alpha-methylacyl-CoA racemase: a new molecular marker for prostate cancer. *Cancer Res*. 2002 Apr 15; 62(8):2220-6.
9. Zhou M, *et al.* Alpha-Methylacyl-CoA Racemase A Novel Tumor Marker Over-expressed in Several Human Cancers and Their Precursor Lesions. *Am J Surg Pathol*. 2002 Jul; 26(7):926-31.
10. Wu CL, *et al.* Analysis of  $\alpha$ -Methylacyl-CoA Racemase (P504S) Expression in High-Grade Prostatic Intraepithelial Neoplasia. *Hum Pathol*. 2004 Aug; 35(8):1008-13.
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