CK5/14 + p63 + P504S

Prediluted Multiplex Antibody Reagent
901-225DS-092921

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Predilute	API 225DS AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 225DS T60	60 tests	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
CK5/14 + p63 + P504S is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of CK5/14, p63 and P504S proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

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 α-methylacyl coenzyme A racemase (AMACR), is a peroxisomal and mitochondrial enzyme that plays a role in bile acid synthesis and β-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 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:
CK5/14 + 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.

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 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human
Positive Tissue Control: Normal prostate and prostatic adenocarcinoma

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1
Pretreatment: Perform heat retrieval using 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 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.
Chromogen (1): Incubate for 5 minutes at RT with Betazoid DAB.
Chromogen (2): Incubate for 5-7 minutes at RT with 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.
Technical Note:
This antibody, for manual use, has been standardized with Biocare's MACH 2 Double Stain 2 detection system. Use TBS for washing steps.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

IVD CE

Rev: 062117

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI225DS is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: CK5/14 p63 P504S
Protocol Template (Description): Multiplex 2 Template 1
Dewaxing (DS Buffer Option): DS2-50
Antigen Retrieval (AR Option): AR1, high pH; 105°C
Block Option: Buffer
Reagent Name, Time, Temp.: CK5/14 p63 P504S, 45 min., 37°C

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

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_3) 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 into 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.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

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.

References Cont'd:

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 αmethylacyl-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. α-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 Overexpressed 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 α-Methylacyl-CoA Racemase (P504S) Expression in High-Grade Prostatic Intraepithelial Neoplasia. Hum Pathol. 2004 Aug; 35(8):1008-13.
11. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
12. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Biocare Medical
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Pacheco, CA 94553
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