

CK5/14 + p63 +P504S

Prediluted Multiplex Antibody
902-225DS-012026



Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Predilute	PPM 225DS AA, H	6.0, 25 mL	Ready-to-use	N/A
NeoPATH PRO	NPAR 225DS T40, T80	40, 80 tests	Ready-to-use	N/A
intelliPATH	IPR 225DS G10	10 mL	Ready-to-use	N/A

Intended Use:

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

Background Information:

CK5 and CK14 are high molecular weight keratins expressed in the cytoplasm of basal cells and myoepithelium of breast tissue.⁵ p63 is a transcription factor present in the nuclei of myoepithelial cells.⁶ P504S is a peroxisomal and mitochondrial enzyme that plays a role in bile acid synthesis and β -oxidation of branched chain fatty acids.⁷

Known Applications:

Immunohistochemistry (Formalin-fixed paraffin-embedded tissues). Other applications have not been tested.

Supplied As:

Buffered saline solution, pH 7.2-7.4, contains a protein carrier and less than 0.1% sodium azide preservative. See Safety Data Sheet for additional details.

Materials and Methods:

Reagents Provided:

CK5/14 + p63 + P504S is provided as follows:

Antibody:	anti-CK5	anti-CK14	anti-p63	anti-P504S
Host Source:	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal
Species Reactivity:	Human. Other species not tested			
Clone:	XM26	LL002	4A4	N/A
Isotype:	IgG1/kappa	IgG3	IgG2a/kappa	IgG
Protein Concentration:	Lot specific Ig concentration is not available.			
Specificity:	CK5	CK14	p63	P504S
Cellular Localization:	Cytoplasmic	Cytoplasmic	Nuclear	Granular cytoplasm
Staining:	Brown (DAB)	Brown (DAB)	Brown (DAB)	Red (Warp Red)

Reconstitution, Mixing, Dilution and Titration:

Prediluted antibody reagent is optimally diluted for use with the above listed staining systems. Further dilution may result in loss of antigen staining. The user must validate any such change. Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results necessitating regular performance of in-house controls.

Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the vial label when stored under these conditions. Do not use after expiration date. Storage under any condition other than those specified must be verified. Diluted reagents should be used promptly; store any remaining reagent at 2°C to 8°C. The stability of user diluted reagents has not been established by Biocare.

Staining Protocol Recommendations (NeoPATH PRO):

NPAR225DS is compatible for use with the NeoPATH PRO. Below are programming and protocol recommendations to assist the user when staining using Biocare's NeoPATH PRO Automated Staining Platform for research applications. The user is responsible for further optimizations of the protocol.

Antibody Protocol: CK5/14+p63+P504S, 20 min at RT

Template: MX2_HIGH_105C_20MINAB_STD

Dewax: Dewax STD; 20 min at 75°C.

Antigen Retrieval (HIER Option): HIGH_105C_30MIN

Enzyme: N/A

Block Option: N/A

Detection: MX2_20AB_STD (Multiplex 2; 30 min at RT)

Chromogen (1): 7 min DAB + 2 min DAB Enhancer at RT

Chromogen (2): 15 min at RT

Hematoxylin: 7 min at RT

Staining Protocol Recommendations (intelliPATH FLX® and manual use):

Below are programming and protocol recommendations to assist the user when staining manually and/or using Biocare's intelliPATH FLX Automated Staining Platform for research applications. The user is responsible for further optimizations of the protocol.

Peroxide Block: Block for 5 minutes with a peroxide blocking reagent.

Pretreatment: Perform heat retrieval if applicable. When using automated equipment, refer to the equipment operator's manual for specific equipment use instructions.

Protein Block: Incubate for 5-10 minutes at RT with a protein blocking reagent.

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

Double Stain Detection: Incubate for 30 minutes at RT using a secondary double stain detection.

Chromogen (1): Incubate for 5 minutes at RT with an HRP chromogen.

Chromogen (2): Incubate for 5-7 minutes at RT with an AP chromogen.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Bluing Solution for 1 minute. Rinse with deionized water.

intelliPATH FLX Automated Slide Stainer:

IPR225DS is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following pretreatment.

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 Double Stain 2 detection system. 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 (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon



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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)¹

2. Handle materials of human or animal origin as potentially biohazardous and dispose of such materials with proper precautions. In the event of exposure, follow the health directives of the responsible authorities where used.^{2,3}

3. 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.⁴

4. Microbial contamination of reagents may result in an increase in nonspecific staining.

5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

6. Do not use reagent after the expiration date printed on the vial.

7. To prevent evaporation and ensure maximum test capacity, promptly cap and remove reagents from automated instruments after each run. Leaving reagents exposed can reduce their effectiveness and the number of tests they can provide. Always store reagents as directed to maintain their integrity.

8. Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of it (or have them treated and disposed of) in accordance with any applicable regulations.

9. Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product

10. The SDS is available upon request and is located at <http://biocare.net>.

11. Report any serious incidents related to this device by contacting the local Biocare representative and the applicable competent authority of the Member State or country where the user is located.

Technical Support:

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

References:

1. 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."

2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.

3. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

4. 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.

5. 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.

6. 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.

7. Ferdinandusse S, *et al.* Subcellular localization and physiological role of α -methylacyl-CoA racemase. J Lipid Res. 2000 Nov; 41(11):1890-6.



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