CK5/14 + p63 + CK7/18

Predilute Multiplex Antibody Reagent

Control Number: 902-360VP-090517

VP Echelon™ Series

Catalog Number: **AVR 360DSK G**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Intended Use:

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

Summary and Explanation:

CK5/14 + p63 + CK7/18 is comprised of mouse monoclonal anti-CK5, anti-CK14, and anti-p63 antibodies and rabbit monoclonal anti-CK7 and anti-CK18 antibodies. CK5 and CK14 are high molecular weight keratins expressed in the cytoplasm of basal cells and myoepithelium of breast tissue (1-4). p63 is a transcription factor present in the nuclei of myoepithelial cells (2,4). In contrast, CK7 and CK18 are low molecular weight cytokeratins primarily expressed in luminal cells of the breast (1-3).

CK5, CK14, p63, CK7 and CK18 have routinely been used as a panel of IHC markers to complement morphological evaluation in the assessment of breast lesions, due to the differential expression of the luminal vs. basal and myoepithelial markers (1-5). Cases of usual ductal hyperplasia (UDH) have been associated with expression of the basal cell markers, intermixed with cells expressing the keratins of luminal cells (1-2, 6-10). Most cases of atypical ductal hyperplasia (ADH) and low grade ductal carcinoma *in situ* (LG-DCIS) were negative for the basal markers and exhibited an immunophenotype indicative of luminal cells (1,5-8). Additionally, the basal phenotype has been shown to be characterized by luminal expression of the basal and myoepithelial markers, using a cocktail of CK5, CK14 and p63 (11-13).

IHC, using CK5, CK14, p63, CK7 and CK18 antibodies, evaluated in combination with hematoxylin and eosin (H&E), has been shown to significantly increase inter-observer agreement amongst pathologists, compared to H&E alone (14).

Principle of Procedure:

A sequential double stain is used for the simultaneous detection of two different antigens within one tissue section. A primary antibody is applied to the tissue, followed by a horseradish peroxidase (HRP) detection system. A denaturing step is required to eliminate cross-reactivity from the application of the second detection system. A second primary antibody is then applied, followed by an alkaline phosphatase (AP) detection system. Visualization of antigens is achieved with DAB and Red chromogens.

Reagent Provided:

CK5/14 + p63 + CK7/18 (VP360DSK) is provided as a prediluted cocktail of anti-CK5, anti-CK14, and anti-p63 mouse monoclonal antibodies (AVR401G), and a cocktail of anti-CK7 and anti-CK18 rabbit monoclonal antibodies (AVR402G), in buffer with carrier protein and preservative.

Antibody	anti-CK5	anti-CK14	anti-p63	anti-CK7	anti-CK18
Clone	XM26	LL002	4A4	BC1	EP30*
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG3	IgG2a/kappa	IgG	IgG
Epitope/ Antigen	CK5	CK14	p63	CK7	CK18
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear	Cytoplasmic	Cytoplasmic
Staining	Brown (DAB)	Brown (DAB)	Brown (DAB)	Red (AP Red)	Red (AP Red)

*Previously known as E431-1

Storage and Stability:

Store at 2ºC to 8ºC. 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

Positive Tissue Control: Breast cancer

Staining Protocol Recommendations:

Using ***ultra*VIEW™ Detection Kit:**

Pretreatment Solution (recommended): CC1

Pretreatment Protocol: Standard

Primary Antibody Cocktail: (AVR401): Incubate for 32 minutes at 37°C.

-ultra Wash-

Denaturation: Incubate for 4 minutes at 90°C

Primary Antibody Cocktail (AVR402): Incubate for 32 minutes at 37°C.

Technical Note:

Biocare's VP-Echelon Series of predilutes have been developed for use with Ventana® Medical Systems, BenchMark® XT Immunohistochemistry Staining System in combination with Ventana® Detection Kits and Ventana® Prep Kit Dispensers.

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_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) (15)
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. (16)
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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

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

CK5/14 + p63 + CK7/18

Predilute Multiplex Antibody Reagent

Control Number: 902-360VP-090517

References:

1. Hicks DG. Immunohistochemistry in the Diagnostic Evaluation of Breast Lesions. Appl Immunohistochem Mol Morph. 2011; 19:501-5.
2. Moriya T, *et al.* New trends of immunohistochemistry for making differential diagnosis of breast lesions. Med Mol Morphol. 2006; 39:8-13.
3. Yeh IT, Mies C. Application of Immunohistochemistry to Breast Lesions. Arch Pathol Lab Med. 2008; 132:349-57.
4. Lerwill MF. Current Practical Applications of Diagnostic Immunohistochemistry in Breast Pathology. Am J Surg Pathol. 2004; 28:1076-91.
5. Moriya T, *et al.* Usefulness of immunohistochemistry for differential diagnosis between benign and malignant breast lesions. Breast Cancer. 2009; 16:173-8.
6. Otterbach F, *et al*. Cytokeratin 5/6 immunohistochemistry assists in differential diagnosis of atypical proliferations of the breast. Histopathology. 2000; 37:232-40.
7. Lacroix-Triki M, *et al.* Value of cytokeratin 5/6 immunostaining using D5/16 B4 antibody in the spectrum of proliferative intraepithelial lesions of the breast. A comparative study with 34betaE12 antibody. Virchows Arch. 2003; 442:548-54.
8. Boecker W, *et al*. Usual ductal hyperplasia of the breast is a committed stem (progenitor) cell lesion distinct from atypical ductal hyperplasia and ductal carcinoma in situ. J Pathol. 2002; 198:458-67.
9. Koo JS, *et al.* Comparison of Immunohistochemical Staining in Breast Papillary Neoplasm of Cytokeratin 5/6 and p63 in core Needle Biopsies and Surgical Excisions. Appl Immunohistochem Mol Morph. 2012; 20:108-15.
10. Ichihara S, *et al.* Double immunostaining with p63 and high-molecular-weight cytokeratins distinguishes borderline papillary lesions of the breast. Path Int. 2007; 57:126-32.
11. Livasy CA, *et al.* Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol. 2006; 19:264-71.
12. Laakso M, *et al.* Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. Mod Pathol. 2005; 18:1321-8.
13. Bhargava R, *et al.* CK5 is More Sensitive than CK5/6 in Identifying the “Basallike” Phenotype of Breast Carcinoma. Am J Clin Pathol. 2008; 130:724-30.
14. Jain RK, *et al.* Atypical ductal hyperplasia: interobserver and intraobserver variability. Mod Pathol. 2011; 24:917-23.
15. 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."
16. 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

60 Berry Drive

Pacheco, CA 94553

USA

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