CINtec® p16 Histology

REF 805-4713

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825-4713

06695256001

IVD Σ 50

Σ 250

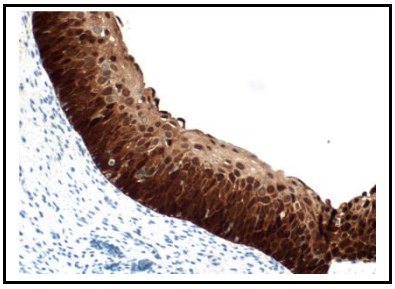

Figure 1. CINtec p16 Histology staining of cervical squamous epithelial cells.

INTENDED USE

CINtec p16 Histology is an immunohistochemistry assay for the qualitative detection of the $p16^{INK4a}$ protein on formalin-fixed, paraffin-embedded tissue sections prepared from cervical biopsies. It is indicated to be used in conjunction with H&E stained slides prepared from the same cervical tissue specimen as an aid to increase diagnostic accuracy and inter-observer agreement in the diagnosis of high-grade cervical intraepithelial neoplasia.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

CINtec p16 Histology consists of a single component: anti-$p16^{INK4a}$ (E6H4), a mouse monoclonal primary antibody.

As a cyclin-dependent kinase inhibitor, $p16^{INK4a}$ plays a key role in cell cycle progression and cellular differentiation.[1,2,3] The $p16^{INK4a}$ protein is part of the retinoblastoma protein (pRB)-mediated control of the G1-S-phase transition and triggers cell cycle arrest in the course of the cellular differentiation process.[1,4] In normal, terminally differentiated cells, $p16^{INK4a}$ is expressed at low levels, typically not detectable by immunohistochemistry.[1,5] However, in some tumor entities, overexpression of $p16^{INK4a}$ protein has been demonstrated to contribute to cell cycle deregulation and cellular transformation. Research studies have identified strong over-expression of $p16^{INK4a}$ in pre-cancerous and cancerous tissues to be closely linked at the molecular level to E7 oncoprotein expression from the human papillomavirus.[1,6]

PRINCIPLE OF THE PROCEDURE

Anti- $p16^{INK4a}$ (E6H4) is a mouse monoclonal primary antibody produced against the $p16^{INK4a}$ protein. Anti- $p16^{INK4a}$ (E6H4) binds to $p16^{INK4a}$ protein in paraffin-embedded tissue sections and exhibits a nuclear and/or cytoplasmic staining pattern. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700; 06396500001) or *ultra*View Universal DAB Detection Kit (Cat. No. 760-500; 05269806001). Refer to the OptiView DAB IHC Detection Kit or *ultra*View Universal DAB Detection Kit package inserts for further information.

REAGENT PROVIDED

CINtec p16 Histology (805-4713) contains sufficient reagent for 50 tests.

One VENTANA 5 mL dispenser of CINtec p16 Histology contains approximately 5.0 µg of a mouse monoclonal antibody.

CINtec p16 Histology (825-4713) contains sufficient reagent for 250 tests.

One VENTANA 25 mL dispenser of CINtec p16 Histology contains approximately 25.0 µg of a mouse monoclonal antibody.

CINtec p16 Histology is diluted in 0.05M Tris-HCl with 1% carrier protein, and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10.0 mg/mL. Specific antibody concentration is approximately 1.0 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CINtec p16 Histology is a mouse monoclonal antibody purified from cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

STORAGE

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and VENTANA BenchMark XT, BenchMark GX and BenchMark ULTRA automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.[7] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark XT, BenchMark GX and BenchMark ULTRA automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CINtec p16 Histology with *ultra*View Universal DAB Detection Kit on a BenchMark GX instrument, BenchMark XT instrument or BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Antibody (Primary)	BenchMark GX instrument 24 minutes, 37°C BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 20 minutes, 36°C
Ultrablock (Antibody Diluent, P/N 251-018; 05261899001)	BenchMark GX instrument 8 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for CINtec p16 Histology with OptiView DAB IHC Detection Kit on a BenchMark GX instrument, BenchMark XT instrument or BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	BenchMark GX instrument Cell Conditioning 1, 32 minutes BenchMark XT instrument Cell Conditioning 1, 48 minutes BenchMark ULTRA instrument Cell Conditioning 1, 48 minutes
Pre Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	BenchMark GX instrument 8 minutes, 37°C BenchMark XT instrument 8 minutes, 37°C BenchMark ULTRA instrument 12 minutes, 36°C
Post-Fixative (Antibody Diluent, P/N 251-018; 05261899001)	BenchMark GX instrument 8 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[8]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are normal pancreas, normal tonsil and cervical carcinoma.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CINtec p16 Histology is nuclear and/or cytoplasmic.

Overexpression of the $p16^{INK4a}$ biomarker within cervical biopsy specimens is represented as a diffuse continuous staining of cells of the basal and parabasal cell layers of the cervical squamous epithelium, with or without staining of the intermediate or intermediate to superficial cell layers. This continuous, diffuse staining pattern should be considered positive. Focal staining is represented by non-continuous staining of isolated cells or small cell clusters, particularly not of the basal and parabasal cells. Focal staining represents a negative staining result.

SPECIFIC LIMITATIONS

CINtec p16 Histology may demonstrate fibroblast and columnar epithelial staining in cervical tissues, which does not interfere with interpretation.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 3 and Table 4 and in the Repeatability section.

Specificity

Table 3. Specificity of CINtec p16 Histology was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	3/3
Adrenal gland	2/3	Lung	0/6
Ovary	0/3	Heart	0/3
Pancreas	3/3	Esophagus	0/3
Parathyroid gland	0/1	Stomach	0/3
Hypophysis	3/3	Small intestine	0/3
Testis	0/3	Colon	0/3
Thyroid	0/5	Liver	0/3
Breast	2/3	Salivary gland	2/3
Spleen	3/3	Kidney	0/3
Tonsil	4/4	Prostate	0/3
Uterus	0/3	Cervix	0/46
Skeletal muscle	0/2	Skin	0/3
Nerve (sparse)	0/3		

Sensitivity

Table 4. Sensitivity of CINtec p16 Histology was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	1/1
Atypical meningioma	0/1
Malignant ependymoma	1/1
Malignant oligodendroglioma	0/1
Serous papillary adenocarcinoma	1/1
Ovarian adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1

Pathology	# positive / total cases
Embryonal carcinoma	0/1
Medullary carcinoma	0/1
Papillary carcinoma	0/1
Breast intraductal carcinoma with early infiltrate	1/1
Breast invasive ductal carcinoma	2/2
Diffuse B-cell lymphoma	1/3
Lung small cell undifferentiated carcinoma	1/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	1/1
Esophageal squamous cell carcinoma	1/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	1/1
Gastrointestinal adenocarcinoma	3/3
GIST	3/3
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/2
Leiomyoma	1/1
Endometrial adenocarcinoma	1/1
Endometrial clear cell carcinoma	1/1
Cervical intraepithelial neoplasia (CIN 1)	19/48
Cervical intraepithelial neoplasia (CIN 2)	35/35
Cervical intraepithelial neoplasia (CIN 3)	26/26
Cervical squamous cell carcinoma	31/32
Embryonal rhabdomyosarcoma	1/1
Anal malignant melanoma	1/1
Basal cell carcinoma	1/1
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	1/1
Hodgkin lymphoma	0/1
Large cell anaplastic lymphoma	1/1
Bladder transitional cell carcinoma	0/1
Low grade leiomyosarcoma	1/1
Osteosarcoma	1/1
Spindle cell rhabdomyosarcoma	1/1
Intermediate grade leiomyosarcoma	1/1

Repeatability

Repeatability studies for CINtec p16 Histology were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument, the BenchMark GX instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument, the BenchMark GX instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

1. Sano T, Oyama T, Kashiwabara K, et al. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol. 1998;153:1741-1748.
2. Agoff SN, Lin P, Morihara J, et al. p16(INK4a) expression correlates with degree of cervical neoplasia: a comparison with Ki-67 expression and detection of high-risk HPV types. Modern Pathol. 2003;16:665-673.
3. Negri G, Egarter-Vigl E, Kasal A, et al. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immuncytochemical correlations. Am J Surg Pathol. 2003;27:187-193.
4. Klaes R, Friedrich T, Spitkovsky D, et al. Overexpression of p16(INK4a) as a specific marker for dysplasia and neoplastic epithelial cells of the cervix uteri. Int J Cancer. 2001;92:276-284.
5. Klaes R, Benner A, Friedrich T, et al. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2002;26:1389-1399.
6. Negri G, Vittadello F, Romano F, et al. p16INK4a expression and progression risk of low-grade intraepithelial neoplasia of the cervix uteri. Virchows Arch. 2004;445:616-620.
7. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
8. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

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