


VENTANA anti-p63 (4A4) Mouse Monoclonal Primary Antibody

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IVD  50

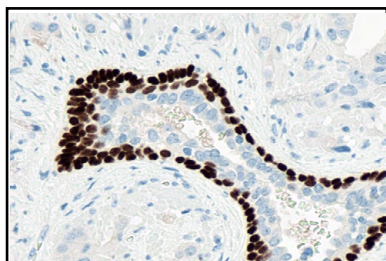


Figure 1. VENTANA anti-p63 (4A4) staining of normal basal cells in prostate tissue.

INTENDED USE

VENTANA anti-p63 (4A4) Mouse Monoclonal Primary Antibody (VENTANA anti-p63 (4A4)) is directed against the p63 protein. This antibody exhibits a nuclear staining pattern and may be used to aid in the differentiation of benign and malignant prostatic or breast lesions, and in the differentiation of lung squamous cell carcinoma and lung adenocarcinoma.

The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

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

The human p63 protein is a member of the p53 family of transcription factors, and has six major isoforms.^{1,2} The p63 protein is highly expressed in the basal or progenitor cells of a variety of epithelial tissues, including those of the prostate, breast, esophagus, bladder, and epidermis,¹ and is important in the development of each of these tissues.^{3,4} In the prostate, p63 can be detected in the basal cells of almost all normal and benign glands,^{5,6} but is not present in the neuroendocrine or luminal, secretory cells.⁴ As basal cells are generally absent from invasive prostate carcinoma, the identification of p63 in normal prostatic gland aids in the identification of prostate carcinoma.^{4,6,7} In high grade prostate intraepithelial neoplasia (PIN), considered a precancerous lesion, dysplastic cells do not express p63, but a residual rim of p63-positive basal cells can be identified.⁴ Similarly, detection of p63 in normal breast myoepithelial cells may be useful in identification of breast neoplasms.⁸ In lung, p63 positivity of 77.8-100% has been reported among squamous cell carcinomas, while the majority of adenocarcinomas (88.7-100%) are negative for this marker.⁹⁻¹² Thus, p63 as part of a panel of biomarkers may aid in the differentiation of lung squamous cell carcinoma and lung adenocarcinoma.

REAGENT PROVIDED

VENTANA anti-p63 (4A4) contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-p63 (4A4) contains approximately 0.7 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCL with 1% carrier protein and ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.140 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-p63 (4A4) is a monoclonal antibody produced as cell culture supernatant material.

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 (*ultraView* Universal DAB Detection Kit), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

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 a VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.¹³ 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

- For *in vitro* diagnostic (IVD) use.
- ProClin 300 is used as a preservative in this solution. 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.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as it may cause incorrect results.
- Consult local and/or state authorities with regard to recommended method of disposal.
- For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on a VENTANA BenchMark Series automated slide stainer in combination with VENTANA detection kits and accessories. A recommended staining protocol for the BenchMark XT and BenchMark ULTRA instrument with *ultraView* Universal DAB Detection Kit is listed in Table 1 and Table 2.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's 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 VENTANA anti-p63 (4A4) with *ultraView* Universal DAB Detection Kit on a BenchMark XT and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 20 minutes, 36°C
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Table 2. Recommended Staining Protocol for VENTANA anti-p63 (4A4) with *VIEW* DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C
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".¹⁴

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are normal prostate and normal tonsil.

STAINING INTERPRETATION

The cellular staining pattern for VENTANA anti-p63 (4A4) is nuclear.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on a BenchMark XT in combination with *ultraView* Universal DAB Detection Kit but the user must validate results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and reproducibility were conducted using VENTANA anti-p63 (4A4) with *ultraView* Universal DAB Detection Kit on BenchMark XT and BenchMark ULTRA instruments.

Specificity

Table 3. Specificity of VENTANA anti-p63 (4A4) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	2/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/13
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	1/3
Parathyroid gland	0/3	Stomach	0/3
Hypophysis	0/3	Small intestine	0/3
Testis	2/3	Colon	0/3
Thyroid	0/3	Liver	0/3
Breast	31/31	Salivary gland	3/3
Spleen	0/3	Kidney	0/3
Tonsil	3/3	Prostate	80/87
Endometrium	0/3	Cervix	3/3
Skeletal muscle	0/3	Skin	3/3
Nerve (sparse)	0/3	Mesothelium and lung	0/3

Sensitivity

Table 4. Sensitivity of VENTANA anti-p63 (4A4) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	1/1
Atypical meningioma	1/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Serous papillary adenocarcinoma	0/1
Mucinous papillary adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	1/1
Seminoma	0/1
Embryonal carcinoma	0/1
Medullary carcinoma	0/1
Papillary carcinoma	0/1
Breast invasive ductal carcinoma	0/1
Diffuse B-cell lymphoma	0/1
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	131/150

Pathology	# positive / total cases
Lung adenocarcinoma	12/117
Esophageal squamous cell carcinoma	1/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	0/1
Gastrointestinal adenocarcinoma	0/3
GIST	0/3
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	2/71
Prostatic transitional cell carcinoma	3/3
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	1/2
Embryonal rhabdomyosarcoma	1/1
Anal malignant melanoma	0/1
Basal cell carcinoma	1/1
Squamous cell carcinoma	1/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	0/1
Diffuse malignant lymphoma	2/3
Hodgkin's lymphoma	0/1
Bladder transitional cell carcinoma	1/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Prostatic leiomyosarcoma	0/3
Prostatic hyperplasia	69/75
Breast hyperplasia	10/10
Lobular carcinoma in situ of the breast	0/4
Ductal carcinoma in situ of the breast	0/8
Infiltrating ductal carcinoma of the breast	0/13
Infiltrating lobular carcinoma of the breast	0/8

Repeatability

Repeatability studies for VENTANA anti-p63 (4A4) 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 and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT and BenchMark ULTRA instruments.

Compatibility

VENTANA anti-p63 (4A4) demonstrated compatibility testing with *ultraView* Universal DAB Detection Kit and MIEV DAB Detection Kit on BenchMark XT and BenchMark ULTRA instruments.

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