

VENTANA anti-E-cadherin (36) Mouse Monoclonal Primary Antibody

REF 790-4497

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IVD  50

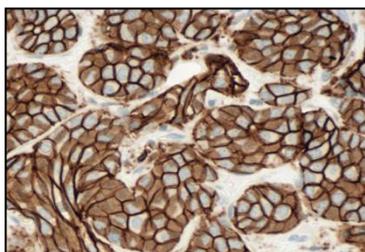


Figure 1. VENTANA anti-E-cadherin (36) Staining of Ductal Carcinoma

of in-situ and/or invasive lobular carcinoma from in situ and/or invasive ductal carcinoma of the breast. This antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

Test results 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

E-cadherin is a 120 kd cell adhesion protein associated with the cytoplasmic membrane of epithelial cells that functions as a tumor suppressor.¹ E-cadherin, along with catenins, function as a calcium-dependent regulator of tight cell-cell adhesion in epithelial cells.² In tumors, aberrations in E-cadherin result in the loss of cytoplasmic membrane localization of the protein and correlate with loss of adhesive properties and an increase in invasive and metastatic potential.³ Antibodies to E-cadherin have been applied to a variety of neoplasms, with breast and lung applications being the most widely published.

In breast, normal ductal cells strongly express E-cadherin in the cytoplasmic membrane. In tumors of ductal origin, the pattern of E-cadherin membrane staining persists, however, tumors of lobular origin frequently exhibit aberrant E-cadherin distribution, with a high frequency having cytoplasmic or no E-cadherin reactivity.^{4,5} Some studies have indicated that lobular breast carcinoma is E-cadherin negative, while ductal is positive, which likely varies with the sample cohort being evaluated and the E-cadherin clone utilized.⁶⁻⁸ While many lobular breast carcinomas do not stain with E-cadherin clone 36, cytoplasmic staining can be observed along with weak, partial, and punctate membrane staining. Additionally, E-cadherin clone 36 has been shown in the literature to demonstrate a nuclear staining pattern in several tumor types.⁹ Since the clinical significance of nuclear E-cadherin staining is not fully known, the pathologist should note the presence of this staining pattern and exercise caution when interpreting E-cadherin stained slides. Only the membranous staining pattern should be relied upon for clinical information at this time, all other staining patterns should be regarded as aberrant.

In lung, E-cadherin has been suggested as useful in distinguishing mesothelioma from adenocarcinoma, with adenocarcinoma being positive and many mesotheliomas being negative. Spindle cell mesotheliomas are primarily negative for E-cadherin while 48% of epithelioid mesotheliomas show reactivity for E-cadherin.¹⁰

REAGENT PROVIDED

VENTANA anti-E-cadherin (36) contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-E-cadherin (36) contains approximately 1.57 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.1 M Phosphate Buffer with 0.3% carrier protein and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 0.314 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-E-cadherin (36) is a monoclonal antibody produced from tissue 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.

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 and BenchMark ULTRA automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.¹¹ 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. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. 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 VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers 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. A recommended staining protocol for the BenchMark XT instrument with *View* DAB Detection Kit is listed in Table 2.

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 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-E-cadherin (36) with *ultraView* Universal DAB Detection Kit on a BenchMark XT and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 24 minutes, 36°C
Optional procedure (ex. Wash, Amplification, etc.)	None
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Table 2. Recommended Staining Protocol for VENTANA anti-E-cadherin (36) with *iView* DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C
Optional procedure (ex. Wash, Amplification, etc.)	None
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".¹²

POSITIVE TISSUE CONTROL

An example of positive control tissue for this antibody is ductal breast carcinoma. Positive staining in tumor cells should be similar to normal expression level observed in ducts of the breast.

STAINING INTERPRETATION / EXPECTED RESULTS

The expected cellular staining pattern for VENTANA anti-E-cadherin (36) is membranous. Cytoplasmic and nuclear staining is considered aberrant and should be interpreted as described in the 'Summary and Explanation' section of this document.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on a BenchMark XT instrument and a 24 minute incubation time on a BenchMark ULTRA instrument 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-E-cadherin (36) with *ultraView* Universal DAB Detection Kit on BenchMark XT and BenchMark ULTRA instruments.

Specificity

Table 3. Specificity of VENTANA anti-E-cadherin (36) 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)	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	3/3	Esophagus	3/3
Parathyroid gland	3/3	Stomach	3/3
Hypophysis	3/3	Small intestine	3/3
Testis	0/3	Colon	2/3
Thyroid	3/3	Liver	3/3
Breast	3/3	Salivary gland	3/3
Spleen	3/3	Kidney	3/3
Tonsil	3/3	Prostate	3/3
Endometrium	3/3	Cervix	2/2
Skeletal muscle	0/3	Skin	3/3
Nerve (sparse)	0/3	Mesothelium and lung	0/3

Sensitivity

Table 4. Sensitivity of VENTANA anti-E-cadherin (36) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

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

Pathology	# positive / total cases
Papillary carcinoma	1/1
Breast intraductal carcinoma	25/28
Breast lobular carcinoma <i>in situ</i>	1/1
Breast invasive ductal carcinoma	165/192
Breast invasive lobular carcinoma	41/57*
Diffuse B-cell lymphoma	0/1
Lung small cell undifferentiated carcinoma	1/17
Lung squamous cell carcinoma	49/54
Lung adenocarcinoma	38/43
Esophageal squamous cell carcinoma	1/1
Esophageal adenocarcinoma	1/1
Gastric mucinous adenocarcinoma	0/1
Gastrointestinal adenocarcinoma	1/1
GIST	0/1
Hepatocellular carcinoma	1/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/1
Prostatic transitional cell carcinoma	1/1
Leiomyoma	0/1
Endometrial adenocarcinoma	1/1
Endometrial clear cell carcinoma	1/1
Uterine squamous cell carcinoma	2/2
Embryonal rhabdomyosarcoma	0/1
Basal cell carcinoma	1/1*
Squamous cell carcinoma	1/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	1/1
Diffuse malignant lymphoma	0/1
Hodgkin's lymphoma	0/1
Diffuse malignant 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	1/1*

Pathology	# positive / total cases
Malignant melanoma	0/1

*Cytoplasmic staining or focal membranous staining

Inter-lot Reproducibility

Determined by testing 3 lots across 1 multi-tissue block (3 tissues per block) on a BenchMark XT instrument. All 3 lots scored equivalently.

Inter-run Repeatability

Determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 50 slides on a BenchMark XT instrument over a 5 day non-consecutive period. 150 out of 150 samples tested scored equivalently.

Intra-run Repeatability

Determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 28 slides on a BenchMark XT instrument. 84 out of 84 samples tested scored equivalently.

Intra-platform Repeatability

Determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 10 slides on 3 BenchMark XT instruments. 90 out of 90 samples tested scored equivalently.

Determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 samples tested scored equivalently.

Inter-platform Repeatability

Determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark ULTRA and 3 BenchMark XT instruments. 45 out of 45 samples tested scored equivalently.

Compatibility Results

VENTANA anti-E-cadherin (36) demonstrated compatibility with VENTANA BenchMark XT, and BenchMark ULTRA instruments and iView DAB Detection Kit and *ultraView* Universal DAB Detection Kit.

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