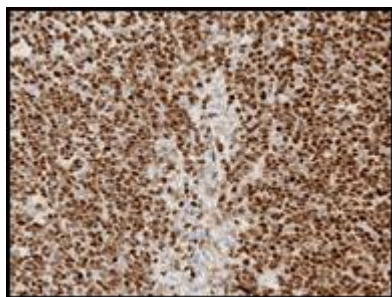


## VENTANA anti-Cyclin D1 (SP4-R) Rabbit Monoclonal Primary Antibody

**REF** 790-4508

05862949001

**IVD**  50



**Figure 1. VENTANA anti-Cyclin D1 (SP4-R) exhibiting a nuclear staining pattern in mantle cell lymphoma tissue**

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

Cyclin D1 is a 36 KD protein that functions as the regulatory subunit for the Cyclin-Dependent Kinase (CDK) 4 and CDK 6 enzymes. Cyclin D1 is important for the progression of the cell cycle through the G1 and G2 phase, however ceases during the S phase.<sup>1</sup> Cyclin D1 is an established oncogene, important in the development of many cancers, and a useful immunohistochemical marker. Overexpression of Cyclin D1 during cell proliferation is often used to distinguish mantle cell lymphoma from small lymphocytic lymphoma.<sup>2</sup> Mantle Cell Lymphoma (MCL) can be characterized by a chromosomal translocation involving rearrangement of the Cyclin D1 proto-oncogene from chromosome 11 to chromosome 14, resulting in an overexpression of Cyclin D1 mRNA.<sup>2</sup> In addition, overexpression of Cyclin D1 has been observed in a number of tumors such as esophageal,<sup>3,6</sup> gastric,<sup>3</sup> lung,<sup>4,6</sup> bladder,<sup>5</sup> hepatocellular cancers,<sup>6</sup> as well as multiple myeloma<sup>7</sup> and meningiomas.<sup>8</sup> Cyclin D1 also plays a role in the detection of breast carcinoma.<sup>9</sup>

### PRINCIPLE OF THE PROCEDURE

VENTANA anti-Cyclin D1 (SP4-R) is a rabbit monoclonal antibody produced against the C-terminus of a recombinant human Cyclin D1 protein. VENTANA anti-Cyclin D1 (SP4-R) binds to the Cyclin D1 protein in paraffin-embedded tissue sections and exhibits a nuclear 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

VENTANA anti-Cyclin D1 (SP4-R) contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-Cyclin D1 (SP4-R) contains approximately 0.33 µg of a rabbit monoclonal antibody.

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

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.07 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-Cyclin D1 (SP4-R) is a recombinant monoclonal antibody produced from purified 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 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 IHC/ISH series of automated instruments. The recommended tissue fixative is 10% neutral buffered formalin.<sup>10</sup> 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](http://www.ventana.com).

### STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH series of automated instruments 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 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-Cyclin D1 (SP4-R) with OptiView DAB IHC on BenchMark GX, BenchMark XT, and BenchMark ULTRA instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 BenchMark ULTRA instrument: 72 minutes BenchMark XT instrument: 48 minutes BenchMark GX instrument: 72 minutes
Pre-primary peroxidase inhibition	Selected
Antibody (Primary)	BenchMark ULTRA instrument 12 minutes, 36°C BenchMark XT instrument 12 minutes, 37°C BenchMark GX instrument 12 minutes, 37°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

**Table 2.** Recommended Staining Protocol for VENTANA anti-Cyclin D1 (SP4-R) with *ultraVIEW* Universal DAB IHC on BenchMark GX, BenchMark XT, and BenchMark ULTRA instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Antibody (Primary)	BenchMark ULTRA instrument 24 minutes, 36°C BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 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".<sup>11</sup>

#### POSITIVE TISSUE CONTROL

The recommended positive control tissue is normal tonsil. The suprabasal cells of the squamous epithelium should demonstrate a moderate but distinct nuclear staining pattern. Endothelial cells also stain positively with Cyclin D1 and serve as a valuable internal positive control. All mantle zone B-cells and germinal center B-cells should be negative.

#### STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA anti-Cyclin D1 (SP4-R) is nuclear.

#### SPECIFIC LIMITATIONS

This antibody has been optimized for a 12 minute incubation time on a BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit, but the user must validate results obtained with this reagent. Endothelial cell and fibroblast staining was observed in positive and negative cases.

OptiView Detection is generally more sensitive than the *ultraVIEW* Detection system. The user must validate the results obtained with this reagent and detection systems.

#### PERFORMANCE CHARACTERISTICS

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

##### Specificity

**Table 3.** Specificity of VENTANA anti-Cyclin D1 (SP4-R) was determined by testing formalin-fixed, paraffin-embedded normal tissues<sup>a</sup>.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum <sup>b</sup>	1/3	Thymus	0/3
Cerebellum <sup>b,c</sup>	2/3	Bone marrow	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	1/3	Esophagus	3/3
Parathyroid gland	4/4	Stomach	2/3
Hypophysis	0/3	Small intestine	3/3
Testis	2/3	Colon	3/3
Thyroid	0/3	Liver	0/3
Breast	3/3	Salivary gland	3/3
Spleen	0/3	Kidney	3/3
Tonsil	3/3	Prostate	3/3
Endometrium	0/3	Cervix	3/3
Skeletal muscle	0/3	Skin	2/3
Peripheral nerve	0/3	Mesothelium	0/3
Lymph node	1/3	Bladder	3/3

<sup>a</sup> positive cases (excluding cerebrum and cerebellum) demonstrated staining of epithelial cells (predominantly basal/proliferative zone).

<sup>b</sup> positive staining of glial cells

<sup>c</sup> cytoplasmic staining of purkinje cells

##### Sensitivity

**Table 4.** Sensitivity of VENTANA anti-Cyclin D1 (SP4-R) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma with necrosis	1/1
Atypical meningioma	1/1
Malignant ependymoma	1/1
Malignant oligodendroglioma	0/1
Serous adenocarcinoma (ovary)	0/1
Adenocarcinoma (ovary)	1/1
Islet cell tumor	1/1

Pathology	# positive / total cases
Adenocarcinoma (pancreas)	1/1
Seminoma (testis)	0/1
Seminoma with vascular tumor thrombus (sparse)	0/1
Medullary carcinoma (thyroid)	1/1
Papillary carcinoma (thyroid)	1/1
Diffuse B-cell lymphoma	0/1
Small cell undifferentiated carcinoma (lung)	0/1
Squamous cell carcinoma (lung)	1/1
Adenocarcinoma (lung)	1/1
Neuroendocrine carcinoma (esophagus)	0/1
Adenocarcinoma (esophagus)	1/1
Signet-ring cell carcinoma	1/1
Adenocarcinoma (small intestine)	1/1
Stromal sarcoma (small intestine)	0/1
Adenocarcinoma (colon)	1/1
Interstitialoma (peritoneum)	0/1
Adenocarcinoma (rectum)	1/1
Moderate malignant interstitialoma (rectum)	1/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	1/1
Clear cell carcinoma (kidney)	1/1
Adenocarcinoma (prostate)	2/2
Adenocarcinoma (uterus)	1/1
Clear cell carcinoma (uterus)	1/1
Squamous cell carcinoma (cervix)	1/2*
Embryonal rhabdomyosarcoma	1/1
Squamous cell carcinoma of chest wall	1/1
Neuroblastoma	1/1
Malignant mesothelioma	1/1
Transitional cell carcinoma (bladder)	1/1
Low grade malignant leiomyosarcoma (bladder)	0/1
Osteosarcoma of right femur (sparse)	0/1
Spindle cell rhabdomyosarcoma	1/1
Moderate malignant leiomyosarcoma (smooth muscle)	0/1
Hodgkin lymphoma	0/1
B cell lymphoma not otherwise specified	3/20
Marginal zone lymphoma of mucosa associated tissue (MALT)	1/15

Pathology	# positive / total cases
Mantle cell lymphoma	72/82
Small lymphocytic lymphoma	3/6**
T cell lymphoma	0/2
Follicular lymphoma	2/30
Ductal carcinoma in situ (breast)	2/2
Invasive ductal carcinoma (breast)	54/54
Invasive lobular carcinoma (breast)	2/2
Tubular carcinoma (breast)	1/1
Invasive papillary carcinoma (breast)	1/1
Intraductal carcinoma (breast)	2/2

\* moderate mast cell staining

\*\* staining in proliferation centers

### Repeatability

Repeatability studies for VENTANA anti-Cyclin D1 (SP4-R) 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, BenchMark GX instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument, BenchMark GX instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

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#### CONTACT INFORMATION



Ventana Medical Systems, Inc.  
1910 E. Innovation Park Drive  
Tucson, Arizona 85755  
USA

+1 520 887 2155

+1 800 227 2155 (USA)



[www.ventana.com](http://www.ventana.com)



Roche Diagnostics GmbH  
Sandhofer Strasse 116  
D-68305 Mannheim  
Germany