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

REF 790-4508

05862949001

IVD Σ 50

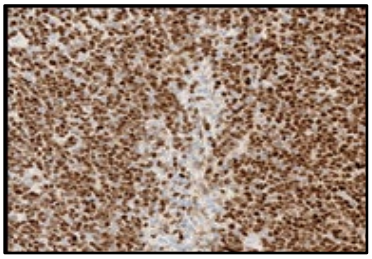

Figure 1. VENTANA anti-Cyclin D1 (SP4-R) antibody exhibiting a nuclear staining pattern in mantle cell lymphoma tissue.

INTENDED USE

VENTANA anti-Cyclin D1 (SP4-R) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of cyclin D1 by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

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

VENTANA anti-Cyclin D1 (SP4-R) Rabbit Monoclonal Primary Antibody (VENTANA anti-Cyclin D1 (SP4-R) antibody) is a rabbit monoclonal antibody produced against the C terminus of a recombinant human cyclin D1 protein. Cyclin D1 is a 36 kDa cell cycle regulatory protein that activates cyclin dependent kinase (CDK) 4 and CDK 6 enzymes.[1,2,3] Cyclin D1 is expressed at higher levels during the G1 phase, the G1/S transition, and G2 phase of the cell cycle, but it is expressed at lower levels during the S phase.[1,2,3] Cyclin D1 overexpression or amplification is associated with tumorigenesis and is detected in many cancers including mantle cell lymphoma, breast carcinoma, lung carcinoma, and gastrointestinal neoplasms.[1,2,3] Of note, mantle cell lymphoma is genetically characterized by the t(11;14)(q13;q32) translocation that juxtaposes the *CCND1* gene to the immunoglobulin heavy chain gene resulting in an overexpression of cyclin D1 protein.[1-5] Detection of cyclin D1 overexpression is often used to distinguish mantle cell lymphoma from chronic lymphocytic leukemia/small lymphocytic lymphoma, which shares many other similar morphological and immunophenotypic features.[5-8] This distinction is important because mantle cell lymphoma is a more aggressive neoplasm.[5-8] In addition to mantle cell lymphoma, a low number of other B-cell lymphoma cases (e.g. plasma cell lymphoma, hairy cell leukemia, diffuse large B-cell lymphoma) express cyclin D1.[4-8]

The detection of cyclin D1 by immunohistochemistry (IHC) with the VENTANA anti-Cyclin D1 (SP4-R) antibody may be used to aid in the diagnosis of mantle cell lymphoma. This antibody may be used as part of a panel of IHC studies. The staining pattern is nuclear.

PRINCIPLE OF THE PROCEDURE

VENTANA anti-Cyclin D1 (SP4-R) antibody binds to the Cyclin D1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections. 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 respective method sheets for further information.

MATERIAL PROVIDED

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

One 5 mL dispenser of VENTANA anti-Cyclin D1 (SP4-R) antibody contains approximately 0.5 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris-HCl with carrier protein and 0.10% ProClin 300 as a preservative.

Specific antibody concentration is approximately 0.1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-Cyclin D1 (SP4-R) antibody is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant material.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and 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 method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 761-700 / 06396500001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 052797710001)
7. Reaction Buffer Concentrate (10X) (Cat No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. General purpose laboratory equipment
15. BenchMark IHC/ISH Instrument

STORAGE AND STABILITY

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 FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[9] Sections should be cut at approximately 4 µm thick and mounted on positively charged glass slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. 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.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[10,11]

8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P273	Avoid release into the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to the tables below 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 User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4508.

Table 2. Recommended staining protocol for VENTANA anti-Cyclin D1 (SP4-R) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 72 minutes	CC1, 48 minutes	ULTRA CC1, 72 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	12 minutes, 37°C	12 minutes, 37°C	12 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for VENTANA anti-Cyclin D1 (SP4-R) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	CC1, Standard	ULTRA CC1 64 minutes, 95°C
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	24 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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."[1,2]

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA anti-Cyclin D1 (SP4-R) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

Examples of positive control tissues for this antibody are 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) antibody is nuclear.

SPECIFIC LIMITATIONS

Endothelial cell and fibroblast staining was observed in positive and negative cases.

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

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of VENTANA anti-Cyclin D1 (SP4-R) antibody was determined by testing FFPE normal tissues.[a]

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum [b]	1/3	Thymus	0/3
Cerebellum [b,c]	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
Pituitary gland	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
Nerve	0/3	Mesothelium	0/3
Lymph node	1/3	Bladder	3/3

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

[b] positive staining of glial cells

[c] cytoplasmic staining of Purkinje cells

Table 5. Sensitivity/Specificity of VENTANA anti-Cyclin D1 (SP4-R) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	0/1
Adenocarcinoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	1/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	0/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	4/4
Invasive ductal carcinoma (Breast)	54/54
Invasive lobular carcinoma (Breast)	2/2
Tubular carcinoma (Breast)	1/1
Invasive papillary carcinoma (Breast)	1/1
Small cell 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 (Stomach)	1/1
Adenocarcinoma (Small intestine)	1/1
Stromal sarcoma (Small intestine)	0/1
Adenocarcinoma (Colon)	1/1
Gastrointestinal stromal tumor (GIST) (Peritoneum)	0/1
Adenocarcinoma (Rectum)	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	1/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	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) [a]	1/2
Embryonal rhabdomyosarcoma (Striated muscle)	1/1
Squamous cell carcinoma (Skin)	1/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	1/1
Osteosarcoma (Bone)	0/1

Pathology	# positive / total cases
Spindle cell rhabdomyosarcoma (Peritoneum)	1/1
Hodgkin lymphoma (Lymph node)	0/1
B cell lymphoma, (Not Otherwise Specified)	3/20
MALT B-cell lymphoma	1/15
Mantle cell lymphoma	72/82
Small lymphocytic lymphoma / chronic lymphocytic leukemia [b]	3/6
Follicular lymphoma	2/30
T cell lymphoma, (NOS)	0/2
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma (Bladder)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

[a] moderate mast cell staining

[b] staining in proliferation centers

Precision

Precision studies for VENTANA anti-Cyclin D1 (SP4-R) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, BenchMark XT and BenchMark ULTRA instrument.
- All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of VENTANA anti-Cyclin D1 (SP4-R) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
E	Updates to Intended Use, Summary and Explanation, Principle of the Procedure, Material Provided, Materials Required but not Provided, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Specific Limitations, Analytical Performance, Clinical Performance, References, Symbols, Intellectual Property, and Contact Information sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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