

CONFIRM anti-Calretinin (SP65) Rabbit Monoclonal Primary Antibody

REF 790-4467

05992184001

IVD  50

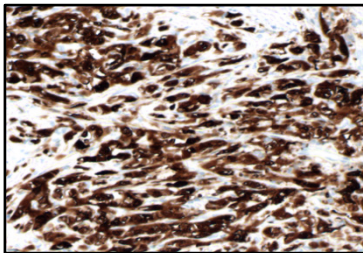


Figure 1. CONFIRM anti-Calretinin (SP65) antibody staining of mesothelioma.

INTENDED USE

CONFIRM anti-Calretinin (SP65) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of calretinin 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

CONFIRM anti-Calretinin (SP65) Rabbit Monoclonal Primary Antibody (CONFIRM anti-Calretinin (SP65) antibody) detects the presence of calretinin expression in several normal and tumor tissue types.¹ Calretinin is a member of a conserved family of calcium-binding proteins that modulates neuron excitability by rapid calcium buffering.^{2,3} In normal tissues, calretinin is expressed in neurons in the brain, the mesothelium, testicular Leydig cells, and ovarian theca lutein and theca interna cells.¹ In tumors, calretinin overexpression is frequently detected in malignant mesothelioma; however, expression is known to vary by subtype.^{4,5} Calretinin expression may be detected in epithelioid and biphasic mesothelioma whereas the sarcomatoid subtype often displays weak or negative calretinin expression.^{6,7,8}

Mesotheliomas and adenocarcinomas of the lung are histologically similar to one another, making the diagnostic process difficult.⁹ The detection of calretinin expression may be used to distinguish mesothelioma (typically calretinin positive) from adenocarcinoma of the lung (typically calretinin negative).^{10,11,12} The detection of calretinin by immunohistochemistry (IHC) with CONFIRM anti-Calretinin (SP65) antibody may be used to aid in the diagnosis of mesothelioma and aid in distinguishing mesothelioma from adenocarcinoma of the lung. This antibody may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Calretinin (SP65) antibody is a rabbit monoclonal antibody produced against human calretinin protein. CONFIRM anti-Calretinin (SP65) antibody binds to the calretinin protein in formalin-fixed, paraffin-embedded (FFPE) tissue. This antibody can be visualized using *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-Calretinin (SP65) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-Calretinin (SP65) antibody contains approximately 30 µg of a rabbit monoclonal (SP65) antibody.

The antibody is diluted in buffered saline containing carrier protein and a preservative.

Specific antibody concentration is approximately 6 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-Calretinin (SP65) antibody is a recombinant rabbit monoclonal antibody produced as Protein A-purified cell culture supernatant.

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. *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Light microscope
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. 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.¹³ Sections should be cut at approximately 4 µm in thickness and mounted on positively charged 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. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
5. Do not use beyond the specified number of tests.
6. 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.
7. 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.

8. 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.^{14,15}
9. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
10. Avoid microbial contamination of reagents as it may cause incorrect results.
11. 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.
12. Consult local and/or state authorities with regard to recommended method of disposal.
13. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
14. 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
	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 to 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 Table 2 for recommended staining protocol.

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 790-4467.

Table 2. Recommended staining protocol for CONFIRM anti-Calretinin (SP65) antibody with *ultraView* Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	ULTRA CC1, 36 minutes, 95 °C
Antibody (Primary)	16 minutes, 37 °C	20 minutes, 36 °C

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS ^a
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."¹⁶

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-Calretinin (SP65) 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 mesothelioma and normal appendix.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Calretinin (SP65) antibody is nuclear and cytoplasmic.

SPECIFIC LIMITATIONS

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 3. Sensitivity/Specificity of CONFIRM anti-Calretinin (SP65) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	3/3	Heart	0/3
Cerebellum	3/3	Esophagus	0/3
Adrenal gland	3/3	Stomach	0/3
Ovary	1/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Parathyroid gland	0/3	Liver	0/3
Pituitary gland	3/3	Salivary gland	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Testis	3/3	Kidney	0/3
Thyroid gland	0/3	Prostate	0/3
Breast	0/3	Endometrium	0/3
Spleen	0/3	Cervix	0/3
Tonsil	0/3	Skeletal muscle	0/3
Thymus	0/3	Skin	0/3
Bone marrow	0/3	Nerve	1/3
Lung	0/6	Mesothelium	10/23
Benign mesothelial proliferation	0/2		

Table 4. Sensitivity/Specificity of CONFIRM anti-Calretinin (SP65) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
Lobular carcinoma in situ (Breast)	0/1
Small cell carcinoma (Lung)	4/8
Large cell carcinoma (Lung)	1/3
Squamous cell carcinoma (Lung)	7/27
Adenocarcinoma (Lung)	4/28
Atypical carcinoid (Lung)	4/6
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	1/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1

Pathology	# positive / total cases
Gastrointestinal stromal tumor (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (Rectum)	0/1
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/1
Urothelial carcinoma (Prostate)	1/1
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	1/1
Spindle cell rhabdomyosarcoma (Peritoneum)	0/1
Lymphoma, NOS	0/3
B-cell lymphoma, NOS	0/1
Hodgkin lymphoma	0/1
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma	0/2
Osteosarcoma (Bone)	0/1
Mesothelioma ^a	35/59

^a Tissue cases assessed consist of epithelioid mesothelioma, biphasic mesothelioma and sarcomatoid mesothelioma.

Precision

Precision studies for CONFIRM anti-Calretinin (SP65) 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 XT and BenchMark ULTRA instrument.
- Between platform precision between the 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 CONFIRM anti-Calretinin (SP65) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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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>

Symbols

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):



Global Trade Item Number



Unique Device Identification



Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
B	Updates to Intended Use, Summary and Explanation, Principle of the Procedure, Material Provided, Material Required but Not Provided, Storage and Stability, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Analytical Performance, Clinical Performance, References, Symbols, Intellectual Property and Contact Information sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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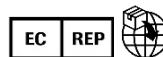
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