

CONFIRM anti-Calretinin (SP65) Rabbit Monoclonal Primary Antibody

REF 790-4467

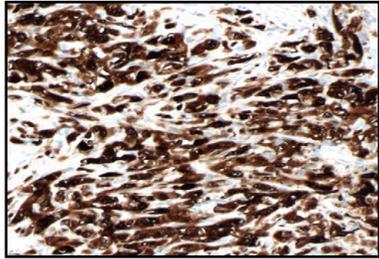


Figure 1. CONFIRM anti-Calretinin (SP65) staining of mesothelioma

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-Calretinin (SP65) Rabbit Monoclonal Primary Antibody (CONFIRM anti-Calretinin (SP65)) is directed against the calcium binding protein, calretinin, expressed by normal and reactive mesothelium, eccrine glands of skin, Sertoli cells of the testis, ovarian stromal cells and adrenal cortical cells. This antibody exhibits a nuclear and cytoplasmic staining pattern and may be used to aid in the identification of

mesothelioma, and in distinguishing mesothelioma from 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

Calretinin, a calcium-binding protein with a molecular weight of 29 kDa, is a member of the family of EF-hand proteins, which also includes S-100 protein. EF-hand proteins are characterized by a helix-loop-helix fold that acts as the calcium-binding site.¹ Calretinin contains six such EF-hand stretches.¹ Calretinin is abundantly expressed in neural tissues.^{2,3} Outside the nervous system, calretinin is found in mesothelial cells, steroid producing cells (including adrenal cortical cells, testicular Leydig cells, ovarian theca interna cells), testicular Sertoli cells, rete testis, ovarian surface epithelium, some neuroendocrine cells, breast glands, eccrine sweat glands, hair follicular cells, thymic epithelial cells, endometrial stromal cells, and fat cells.^{4,5} Calretinin is detected in most malignant mesothelioma and therefore may aid in distinguishing mesothelioma from adenocarcinomas of lung.⁶⁻¹⁰ Other tumors usually found positive for calretinin are adenomatoid tumor, sex cord-stromal tumors (mostly Sertoli-stromal tumors), mesonephric cervical adenocarcinoma, Wolffian adnexal tumor, synovial sarcoma and ameloblastoma.^{5,11-14} Positive calretinin staining has also been noted in some adenocarcinomas including colon and pancreas.¹⁵

REAGENT PROVIDED

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

One 5 mL dispenser of CONFIRM anti-Calretinin (SP65) contains approximately 29.7 µg of a rabbit monoclonal (SP65) antibody.

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

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 5.94 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-Calretinin (SP65) is a Protein A purified recombinant rabbit monoclonal antibody.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles and Procedures, (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 (i.e., *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 automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.¹⁶ 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 use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local and/or state authorities with regard to recommended method of disposal.

STAINING PROCEDURE

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

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 CONFIRM anti-Calretinin (SP65) with *ultraView* Universal DAB Detection Kit on BenchMark XT/ULTRA instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Mild Cell Conditioning 1
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 Reagent, 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".¹⁷

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are mesothelioma and normal appendix.

STAINING INTERPRETATION

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

SPECIFIC LIMITATIONS

This antibody has been optimized on Ventana BenchMark XT and BenchMark ULTRA instruments in combination with *ultraView* Universal DAB Detection Kit (REF 760-500) at a 16 or 20 minute primary incubation time, respectively. However, the user must validate individual laboratory results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

- Specificity of CONFIRM anti-Calretinin (SP65) was determined by testing formalin-fixed, paraffin-embedded normal and neoplastic tissues.
 For normal tissues, results are as follows: (3/3) cerebrum, (3/3) cerebellum, (3/3) adrenal gland, (1/3) ovary, (0/3) pancreas, (0/3) parathyroid gland, (3/3) hypophysis, (3/3) testis, (0/3) thyroid gland, (0/3) breast, (0/3) spleen, (0/3) tonsil, (0/3) thymus, (0/3) myeloid, (0/3) pulmonary, (0/3) myocardium, (0/3) esophagus, (0/3) gastric fundus, (0/3) small intestine, (0/3) colon, (0/3) liver, (0/3) salivary gland, (0/3) kidney, (0/3) prostate, (0/3) endometrium, (0/3) cervix, (0/3) skeletal muscle, (0/3) skin, (1/3) nerve, (2/3) mesothelium and lung.
 For neoplastic tissues, results are as follows: (0/1) glioblastoma, (0/1) atypical meningioma, (1/1) malignant ependymoma, (0/1) malignant oligodendroglioma, (1/2) papillary adenocarcinoma, (0/1) islet cell carcinoma, (1/1) pancreatic adenocarcinoma, (0/1) seminoma of testis, (0/1) embryonal carcinoma of testis, (1/1) medullary carcinoma of thyroid, (0/1) papillary carcinoma of thyroid, (0/3) breast carcinoma, (0/1) diffuse B-cell lymphoma, (0/3) lung carcinoma, (0/1) esophageal squamous cell carcinoma, (1/1) esophageal adenocarcinoma, (0/1) gastric adenocarcinoma, (0/2) intestinal adenocarcinoma, (0/2) colon carcinoma, (0/2) rectal carcinoma, (0/1) hepatocellular carcinoma, (0/1) hepatoblastoma, (0/1) clear cell carcinoma of kidney, (0/1) prostate adenocarcinoma, (1/1) transitional cell carcinoma, (0/1) leiomyoma, (0/1) endometrial adenocarcinoma, (0/1) endometrial clear cell carcinoma, (0/3) squamous cell carcinoma, (0/1) embryonal rhabdomyosarcoma, (0/1) malignant melanoma, (0/1) basal cell carcinoma, (0/1) neurofibroma, (1/1) neuroblastoma, (1/1) malignant mesothelioma, (0/3) diffuse malignant lymphoma, (0/1) Hodgkin's lymphoma, (0/1) transitional cell carcinoma, (0/2) leiomyosarcoma, (0/1) osteosarcoma, (0/1) spindle cell rhabdomyosarcoma, (0/2) nonmalignant mesothelioma, (34/58) malignant mesothelioma, (8/20) normal mesothelioma, (4/27) lung adenocarcinoma, (4/6) atypical carcinoid of lung, (1/3) large cell carcinoma of lung, (4/7) small cell carcinoma of lung, and (7/26) lung squamous cell carcinoma.
- Sensitivity of CONFIRM anti-Calretinin (SP65) was determined by testing a variety of formalin-fixed, paraffin-embedded target specific neoplastic tissues.
- Inter-lot reproducibility was determined by testing 3 lots across 1 multi-tissue block (3 tissues per block) on a BenchMark XT instrument. 3 out of 3 tissue samples tested across all 3 lots scored equivalently.
- Intra-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 14 slides on a BenchMark XT instrument. 84 out of 84 tissue samples tested scored equivalently.
- Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on a BenchMark XT instrument over a 5 day non-consecutive period. 150 out of 150 tissue samples tested scored equivalently.
- Intra-platform reproducibility was determined by staining 2 multi-tissue blocks (3 tissues per block) across 5 slides on 3 BenchMark XT instruments. 90 out of 90 tissue samples tested scored equivalently.
- Intra-platform reproducibility was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 tissue samples tested scored equivalently.

- Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 90 out of 90 tissue samples tested scored equivalently.
- Compatible with BenchMark XT instrument using MIEW DAB Detection Kit.

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