


CONFIRM anti-CD4 (SP35) Rabbit Monoclonal Primary Antibody

REF 790-4423
05552737001

IVD  50

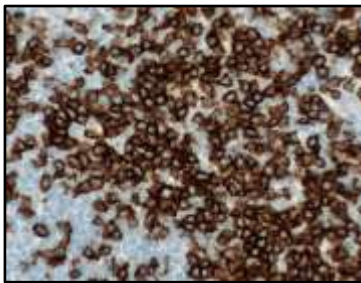


Figure 1. CONFIRM anti-CD4 (SP35) antibody staining of T-cells.

INTENDED USE

CONFIRM anti-CD4 (SP35) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of CD4 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

CD4 is a 55 kDa monomeric type I transmembrane glycoprotein expressed by helper T-cells and at low levels in macrophages and dendritic cells but is absent on B-cells.^{1,2} CD4 acts as a co-receptor for the T-cell receptor and interacts directly with major histocompatibility complex class II molecules on antigen-presenting cells, which stimulate T-cell activation.^{1,2} CD4 is absent on immature T-cells and expressed in later stages of T-cell development as the cell matures into a helper T-cell.¹⁻⁴ T-cell lymphomas derived from helper T-cells (e.g. adult T-cell leukemia, angioimmunoblastic T-cell lymphoma, mycosis fungoides) often retain CD4 expression while CD4 is generally absent in subtypes derived from cytotoxic T-cells or NK cells, such as enteropathy associated T-cell lymphoma, T-lymphoblastic lymphoma, and NK/T-cell lymphoma.¹⁻⁴ Some T-cell lymphomas that exhibit variable CD4 expression include anaplastic large cell lymphoma and peripheral T-cell lymphoma.¹⁻⁴

The detection of CD4 by immunohistochemistry (IHC) with the CONFIRM anti-CD4 (SP35) Rabbit Monoclonal Primary Antibody (CONFIRM anti-CD4 (SP35) antibody), when evaluated with other markers, may be used to aid in the identification of normal helper T-cells and sub-classification of T-cell lymphoma. The cellular staining pattern for CONFIRM anti-CD4 (SP35) antibody is membranous.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD4 (SP35) antibody binds to the CD4 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections. 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-CD4 (SP35) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD4 (SP35) antibody contains approximately 12.5 µg of a recombinant rabbit monoclonal antibody.

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

Specific antibody concentration is approximately 2.5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-CD4 (SP35) antibody is a recombinant rabbit monoclonal antibody produced as 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 Staining, 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. Negative Control Rabbit Ig (Cat. No. 760-1029 / 05266238001)
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. General purpose laboratory equipment
14. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2 to 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.⁵ 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 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. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
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.^{6,7}
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 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 790-4423.

Table 2. Recommended staining protocol for CONFIRM anti-CD4 (SP35) 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, Standard	ULTRA CC1, Standard
Antibody (Primary)	16 minutes, 37°C	32 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 environment conditions, it may be necessary to increase or decrease the primary antibody incubation, and cell conditioning based on individual specimens detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁸

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-CD4 (SP35) 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 and liver. The parafollicular zone cells of the tonsil are generally CD4 positive as are the sinusoids of liver.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-CD4 (SP35) antibody is membranous.

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-CD4 (SP35) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Heart	0/3
Cerebellum	0/3	Esophagus	0/3
Adrenal gland	0/3	Stomach	0/3
Ovary	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Lymph node ^c	7/7	Liver ^a	2/3
Parathyroid gland	3/3	Salivary glands	0/3
Pituitary gland	0/3	Kidney	0/3
Testis	0/3	Prostate	0/3
Thyroid	0/3	Bladder ^b	2/3
Breast	0/3	Endometrium	0/3
Spleen ^c	4/4	Cervix	0/3
Tonsil ^c	5/5	Skeletal muscle	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Thymus	3/3	Skin	0/3
Bone marrow	0/3	Nerve	0/3
Lung	0/3	Mesothelium	0/3

^a sinusoids; ^b urothelium; ^c category includes normal and reactive tissue

Table 4. Sensitivity/Specificity of CONFIRM anti-CD4 (SP35) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Brain)	0/1
Meningioma (Brain)	0/1
Ependymoma (Brain)	0/1
Oligodendroglioma (Brain)	0/1
Serous adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Lobular carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1

Pathology	# positive / total cases
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/1
Urothelial carcinoma (Prostatic urethra)	0/1
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 (Mediastinum)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	0/1
Lymphoma, NOS	1/23
Lymphoma, null type	1/1
Hodgkin lymphoma	0/15
B-Cell Lymphoma, NOS	0/73
Diffuse large B-cell lymphoma (DLBCL)	0/2
MALT B-cell lymphoma	0/7
Mantle cell lymphoma	0/1
Chronic lymphocytic leukemia	0/2
Extranodal marginal zone B-cell lymphoma	0/2
Myeloma (Bone Marrow)	1/1
T-cell lymphoma, NOS	7/8
Peripheral T-cell lymphoma	14/29
Mycosis fungoides	1/1
Enteropathy associated T-cell lymphoma	0/6
Angioimmunoblastic T-cell lymphoma	12/13
Lennert lymphoma	2/3
T lymphoblastic lymphoma	1/2
Anaplastic large cell lymphoma	2/6
NK/T-cell lymphoma, nasal type	1/7
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

Precision

Precision studies for CONFIRM anti-CD4 (SP35) 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-CD4 (SP35) 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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4. Higgins RA, Blankenship JE, Kinney MC. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. Arch Pathol Lab Med. 2008;132(3):441-461.
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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, 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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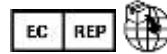
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