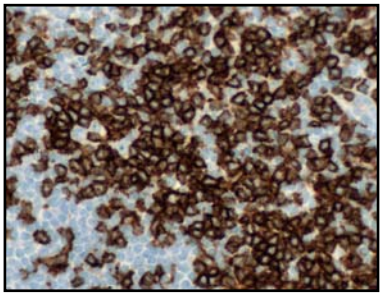
CONFIRM anti-CD4 (SP35) Rabbit Monoclonal Primary Antibody

Catalog Number 790-4423



INTENDED USE

This antibody is intended for *in vitro* diagnostic (IVD) use. Ventana Medical Systems' (Ventana) CONFIRM anti-CD4 (SP35) Rabbit Monoclonal Primary Antibody is intended for the qualitative detection of CD4 in sections of formalin-fixed, paraffin-embedded normal and neoplastic human tissues. CD4 is present on helper-inducer T lymphocytes that recognize antigen in the context of MHC class 2 molecules. CD4 positive staining results may aid in identifying T-cell lymphomas and in identifying the T helper-inducer cell subset of T lymphocytes in normal tissues. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

SUMMARY AND EXPLANATION

CD4 is a single chain transmembrane glycoprotein found on the helper-inducer T cell subset representing roughly 45% of mature peripheral blood T-cell lymphocytes. CD4 is also present on about 80% of thymocytes and at a lower level on monocytes, while B-cells do not express CD4.[1] CD4 acts as a co-receptor for the T cell receptor (TCR) and transduces the TCR signal following interaction with an antigen presenting cell (APC) during T cell activation. CD4 interacts directly with MHC class II molecules on the surface of the APC.[2,3] CD4 serves as receptor for HIV, and CD4 positive cell counts are used to predict prognosis in HIV-infected individuals.[4] Anti-CD4 can be used as part of a panel of antibodies to classify T-cell disorders including T cell lymphomas and to differentiate between helper T cells and killer cells.[5,6]

REAGENT PROVIDED

CONFIRM anti-CD4 (SP35) contains sufficient reagent for staining 50 slides.

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

The antibody is diluted in 0.05 M Tris-HCL with 1% carrier protein and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 2.5 µg/mL. There is no known irrelevant antibody reactivity observed in this product.

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 Preparation, (4) Quality Control, (5) Troubleshooting, (6) Interpretation of Staining, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (for example, *ultra*View Universal DAB detection kit), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2 to 8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, after every use the cap must be replaced and the dispenser must be immediately placed 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.[7] 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 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 BenchMark XT and BenchMark ULTRA series automated slide stainers with *ultra*View Universal DAB detection kit is listed below in Table 1. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument operator manual. Refer to the appropriate Ventana detection package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-CD4 (SP35) with *ultra*View Universal DAB detection kit on BenchMark XT and BenchMark ULTRA series automated slide stainers.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary) Incubation	Approximately 16 Minutes, 37°C for BenchMark XT instrument Approximately 32 Minutes, 36°C for BenchMark ULTRA instrument
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 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]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for CONFIRM anti-CD4 (SP35) are normal tonsil (as depicted in the above image) and liver. The parafollicular zone cells of the tonsil are generally CD4 positive as are the sinusoids of liver.

STAINING INTERPRETATION

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

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on BenchMark XT instruments and a 32 minute incubation time on BenchMark ULTRA instruments in combination with *ultra*View Universal DAB detection kit , however the user must validate individual laboratory results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-CD4 (SP35) was determined by testing formalin fixed, paraffin embedded normal and neoplastic tissues. For normal tissues, results are as follows: adrenal gland (0/3), bone marrow (0/3), brain cerebrum (0/3), brain cerebellum (0/3), breast (0/3), cervix (0/2), colon (0/3), esophagus (0/3), heart (0/3), hypophysis (0/3), small intestine (0/3), kidney (0/3), liver (1/3), lung (0/3), mesothelium (0/3), nerve (0/3), ovary (0/3), pancreas (0/3), parathyroid (3/3), prostate (0/3), salivary gland (0/3), skin (0/3), spleen (3/3), stomach (0/3), striated muscle (0/3), testis (0/3), thymus (2/2), thyroid (0/3), tonsil (3/3), and uterus (0/3). For neoplastic tissues, results are as follows: atypical meningioma (0/1), glioblastoma (0/1), ependymoma (0/1), oligodendroglioma (0/1), ovarian serous papillary adenocarcinoma (0/1), ovarian mucous papillary adenocarcinoma (0/1), islet cell carcinoma (0/1), pancreatic adenocarcinoma (0/1), testicular seminoma and embryonal carcinoma (0/2), medullary thyroid carcinoma (0/1), papillary thyroid carcinoma (0/1), intraductal, lobular, and invasive breast carcinoma (0/3), diffuse B-cell lymphoma in spleen (0/1), small cell undifferentiated carcinoma in lung (0/1), squamous cell lung carcinoma (0/1), lung adenocarcinoma (0/1), esophageal squamous cell and adenocarcinoma (0/2), mucinous adenocarcinoma in stomach (0/1), intestinal adenocarcinoma (0/2), colon adenocarcinoma (0/2), rectal adenocarcinoma (0/2), hepatocellular carcinoma (0/1), hepatoblastoma (0/1), clear cell carcinoma (0/1), adenocarcinoma in prostate (0/1), transitional cell carcinoma in prostate and bladder (0/2), uterine leiomyoma (0/1), endometrial adenocarcinoma (0/1), uterine clear cell and squamous carcinomas (0/3), embryonal rhabdomyosarcoma (0/1), rectal melanoma (0/1), basal cell carcinoma in skin (0/1), squamous cell carcinoma in skin (0/1), neurofibroma and neuroblastoma (0/2), mesothelioma (0/1), Hodgkin's lymphoma (0/1), diffuse type lymphoma (0/3), leiomyosarcoma (0/2), osteosarcoma (0/1), and spindle cell rhabdomyosarcoma (0/1).
2. Sensitivity of CONFIRM anti-CD4 (SP35) was determined by testing a variety of formalin fixed, parrafin embedded target specific neoplastic tissues. Results are as follows: T-cell lymphoma (10/12), B-cell lymphoma (0/102), Hodgkin's Lymphoma (0/14), Mantle cell lymphoma (0/1), Myeloma (1/1), and Normal Lymph Node (4/4)
3. Lot to lot repeatability was determined by testing 3 lots across 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) on a BenchMark XT instrument. 6 out of 6 samples across all 3 lots scored equivalently.
4. 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 five day non-consecutive period. 150 out of 150 samples tested scored equivalently.
5. 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 samples tested scored equivalently.
6. Intra-platform repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block) across 5 slides across 3 BenchMark XT instruments. 90 out of 90 samples tested scored equivalently.
7. Intra-platform repeatability was determined by staining 1 multi-tissue block (3 tissues) across 5 slides across 3 BenchMark ULTRA instruments. 45 out of 45 samples tested scored equivalently.
8. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides across 3 BenchMark XT instrument and 3 BenchMark ULTRA instruments. 90 out of 90 samples tested scored equivalently.
9. Compatible with *ultra*View Universal DAB and *i*VIEW DAB detection kits.

REFERENCES

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