CD4 (RM)

Prediluted Rabbit Monoclonal Antibody
901-3209-060123

BIOCARE MEDICAL

Catalog Number:	**API 3209 AA**	**VLTR 3209 G20**
Description:	6.0 mL, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
CD4 [EP204] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of CD4 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
CD4, a member of the immunoglobulin superfamily, is a transmembrane glycoprotein, expressed on mature thymocytes, T-helper cells, the majority of mature peripheral T cells, and a subset of suppressor or cytotoxic T cells (1). In lymphoid tissue, CD4 expression can be found in the paracortical T zone. CD4+ T cells are also seen scattered in germinal center and mantle zone. CD4 is expressed in the majority of mature T-cell lymphomas (2). CD4 has been used in lymphoma panels that include CD3, CD5, CD8, CD7 and TIA-1 (2-3). A CD4 numerical assessment has been useful in HIV-infected individuals, as HIV infection depletes CD4+ T cells and has a strong association with the level of systemic CD4+ T cell activation (4). Tumor infiltrating CD4+ T cells may also be a prognostic factor for the strategy of early antitumor immunity (5).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Rabbit monoclonal
Species Reactivity: Human; others not tested
Clone: EP204
Isotype: IgG
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: A synthetic peptide corresponding to residues of human CD4 protein
Cellular Localization: Cell membrane
Positive Tissue Control: Tonsil
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative
Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTR3209 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
Enzyme: Incubate for 10 minutes with Val Zyme Pronase (1:25 mix).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Secondary: N/A
Linker: Incubate for 10 minutes with Val Universal Linker.
Polymer: Incubate for 20 minutes with Val Universal Polymer.
Chromogen: Incubate for 5 minutes with Val DAB.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: N/A
Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Performance Characteristics:
Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.
Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.
Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011
Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

Precautions Cont'd:
91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:
1. Leong A S-Y, Cooper K and Leong F J W-M eds. Manual of diagnostic antibodies for immunohistology, second edition 2003. Greenwich Medical Media Ltd: p. 65-6.
2. Izban KF, Hsi ED, Alkan S. Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. Mod Pathol. 1998 Oct; 11(10):978-82.
3. Macon WR, Salhany KE. T-cell subset analysis of peripheral T-cell lymphomas by paraffin section immunohistology and correlation of CD4/CD8 results with flow cytometry. Am J Clin Pathol. 1998 May; 109(5):610-7.
4. Gordon SN, *et al*. Disruption of intestinal CD4+ T cell homeostasis is a key marker of systemic CD4+ T cell activation in HIV-infected individuals. J Immunol. 2010 Nov 1;185(9):5169-79.
5. Rathore AS, *et al*. CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. Indian J Med Res. 2014 Sep;140(3):361-9.
6. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
7. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

Table 1: Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Breast Cancer	0	15
Colon Cancer	0	30
Lung Cancer	0	20
Prostate Cancer	0	15

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	0	3
Cerebellum	0	3
Adrenal	0	3
Ovary	0	3
Pancreas	0	3
Parathyroid	0	3
Pituitary	0	3
Testis	0	3
Thyroid	0	3
Breast	0	3
Spleen	3	3
Tonsil	3	3
Thymus	3	3
Bone Marrow	3	3
Lung	0	3
Heart	0	3
Esophagus	0	3
Stomach	0	3
Small Intestine	0	3
Colon	0	3
Liver	0	3
Salivary Gland	0	3
Kidney	0	3
Prostate	0	3
Uterus	0	3
Cervix	0	3
Skeletal Muscle	0	3
Skin	0	3
Peripheral Nerve	0	3
Linging Cells	0	3

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

IVD CE
Rev: 062117

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080