CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody

REF 790-4341

05278422001

IVD Σ 50

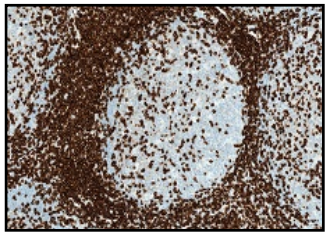

Figure 1. CONFIRM anti-CD3 (2GV6) staining of T cells in lymphoma.

INTENDED USE

Ventana Medical Systems, Inc.'s (Ventana) CONFIRM anti-CD3 (2GV6) (Primary Antibody (CONFIRM anti-CD3 (2GV6)) is a rabbit monoclonal antibody (IgG) directed against the nonglycosylated epsilon chain of the human CD3 molecule. This antibody is intended for use to qualitatively identify T cells by light microscopy in sections of formalin-fixed, paraffin-embedded tissue on a VENTANA automated slide stainer.

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-CD3 (2GV6) is a rabbit monoclonal antibody produced against a synthetic peptide from the carboxy terminal region of the CD3 epsilon chain; and binds to the CD3 epitope in paraffin embedded tissue sections.

REAGENT PROVIDED

CONFIRM anti-CD3 (2GV6) contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD3 (2GV6) contains approximately 2 µg (0.4 µg/mL) of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 2% carrier protein, and 0.10% ProClin 300, a preservative containing the active ingredients 5-chloro-2-methyl-4-isothiazolin-3-one and 2- methyl-4-isothiazolin-3-one. There is a trace of (~2%) fetal calf serum of U.S. origin from the stock solution.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.45 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-CD3 (2GV6) is a rabbit IgG antibody. 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 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 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 BenchMark Series automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[1] 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 (IVD) use.
2. ProClin 300 is used as a preservative in this solution. 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.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on a VENTANA BenchMark Series automated slide stainer in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 for recommended staining protocols.

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'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-CD3 (2GV6) with *i*VIEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark Series instrument 16 minutes, 37°C
A/B Block (Biotin Blocking)	Optional
Amplification	Optional
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for CONFIRM anti-CD3 (2GV6) with *ultra*VIEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	None required,
Antibody (Primary)	BenchMark Series instrument 16 minutes, 37°C
A/B Block (Biotin Blocking)	Optional
Amplification	Optional
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 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".[2]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are tonsil or spleen.

STAINING INTERPRETATION

The positive staining tissue components (T cells) are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and may serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, and not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

SPECIFIC LIMITATIONS

The antibody has been optimized for a 16 minute incubation time in combination with VENTANA detection kits and the VENTANA automated slide stainers. Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change. However, because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[2]

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed below.

Specificity

CONFIRM anti-CD3 (2GV6) specificity was tested across a 60 core normal tissue array that showed no specific cytoplasmic/membrane staining for the following normal tissues: adrenal (0/3)*, brain (0/3), breast (0/3)*, colon (0/2)*, fibroadipose tissue (0/1)*, heart (0/2)*, kidney (0/3)*, large intestine (0/1)*, liver (0/4)*, lung (0/4)*, ovary (0/4)*, pancreas (0/3)*, prostate (0/4)*, skin (0/2)*, small intestine (0/2)*, spleen (0/3)*, stomach (0/3)*, testis (0/3)*, thyroid (0/3)*, tonsil (0/3)* and uterus (0/4)*.

CONFIRM anti-CD3 (2GV6) specificity was also tested across a 50 core neoplastic array that showed no specific cytoplasmic/membrane staining for the following neoplastic tissues: breast (0/4)*, carcinoids (0/2)*, colon (0/3)*, hepatocellular carcinoma (0/2)*, kidney (0/3)*, leiomyoma (0/2)*, liver (0/4)*, lung (0/2)*, non T-cell lymphoma (0/3),* melanoma (0/2)*, ovary (0/2)*, pancreas (0/3)*, prostate (0/3)*, renal cell carcinoma (0/2)*, sarcoma (0/2)*, skin (0/1)*, stomach (0/3)*, teratoma (0/2), thyroid (0/3)*, undifferentiated cancer (0/1)*, and vascular tissue (0/1)*.
*Those tissues above marked with an asterisk are tissues where infiltrating T cells were noted.

Sensitivity

Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens CD3 detection by CONFIRM anti-CD3 (2GV6) and may generate false negative results. Sensitivity was evaluated on an 80 core lymphoma array that consisted primarily of B-cell lymphomas and included ten (10) T-cell lymphomas that showed specific cytoplasmic/membrane staining. Lymphomas in the following tissue types were evaluated: bone (0/2)*, colon (0/14)*, intestine (0/6)*, kidney (0/2)*, larynx (0/2)*, lymph node (0/2)*, mediastinum (4/4), mesentery (0/2)*, nose (2/2), spleen (2/6)*, stomach (0/28)*, striated muscle (0/1)*, thyroid (0/2)*, and tonsil (2/2)*.
*Those tissues above marked with an asterisk are tissues where infiltrating T cells were noted.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper barcode label. If the slide is labeled properly, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step may be included.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific antibody staining is too intense, the run should be repeated with the primary antibody incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
6. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
7. For corrective action, refer to the Step By Step Procedure section of the automated slide stainer Operator's Manual or contact your local Ventana office.

Repeatability

Repeatability studies for CONFIRM anti-CD3 (2GV6) were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument.
- Inter-platform reproducibility between the BenchMark XT instrument.

All studies met their acceptance criteria (≥ 90%).

REFERENCES

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
2. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

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