

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

REF 790-4341

05278422001

IVD  50

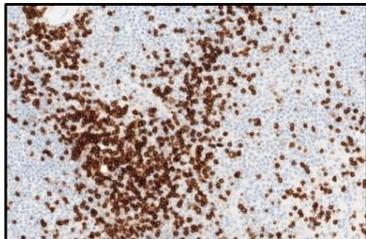


Figure 1. CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody staining of tonsil using OptiView DAB IHC Detection Kit.

INTENDED USE

CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody (CONFIRM anti-CD3 (2GV6) antibody) is a rabbit monoclonal antibody (IgG) directed against the non-glycosylated epsilon chain of the human CD3 molecule.¹ CONFIRM anti-CD3 (2GV6) antibody is intended for use to qualitatively identify T cells by light microscopy in sections of formalin-fixed, paraffin-embedded tissue on a VENTANA BenchMark IHC/ISH series of automated instruments.

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) antibody is a rabbit monoclonal antibody produced against a synthetic peptide from the carboxy-terminal region of the CD3 epsilon chain. CD3 epsilon chain is expressed in T lymphocytes, natural killer (NK) cells, and T-cell and NK-cell neoplasms.¹ The CD3 staining is located primarily in the membrane of mature T-cells; however, it can also be found in the cytoplasm of pro-thymocytes and NK cells.²

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD3 (2GV6) antibody may be used as the primary antibody for immunohistochemical staining of paraffin tissue sections. CONFIRM anti-CD3 (2GV6) antibody exhibits a membranous and/or cytoplasmic staining pattern. CONFIRM anti-CD3 (2GV6) antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

In addition to staining with CONFIRM anti-CD3 (2GV6) antibody, a second slide should be stained with the appropriate negative control reagent.

MATERIAL PROVIDED

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

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

CONFIRM anti-CD3 (2GV6) antibody is diluted in 0.05 M Tris-HCl with 1% carrier protein and ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-CD3 (2GV6) antibody is a rabbit monoclonal antibody produced as a 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 Results, and 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.

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. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, 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 (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.³ 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. 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. 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.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining of any IHC assay (for example, lack of primary antibody or counterstain on the tissue). Ask your Roche representative for a copy of "Impacts of Environmental Stresses on IHC Positively Charged Slides" to better understand how to use these types of slides.
7. 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.^{4,5}
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.

10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument Operator's Manual, and instructions for use of all necessary components.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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
 Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	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, a mixture of: 5-chloro-2-methyl-4-isothiazolin-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 and Table 3 for recommended staining protocols.

CONFIRM anti-CD3 (2GV6) 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 Operator's Manual. Refer to the appropriate VENTANA detection kit 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 790-4341.

Table 2. Recommended staining protocol for CONFIRM anti-CD3 (2GV6) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 40 minutes, 100°C	CC1, 40 minutes, 100°C	ULTRA CC1, 40 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	20 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		

Procedure Type	Method		
	GX	XT	ULTRA
Post Counterstain	Bluing, 4 minutes		

Table 3. Recommended staining protocol for CONFIRM anti-CD3 (2GV6) antibody with *ultraView* Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA CC1, Mild
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	20 minutes, 36°C
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."⁶

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 spleen, tonsil, or lymph node.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-CD3 (2GV6) antibody is membranous and/or cytoplasmic.

SPECIFIC LIMITATIONS

The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes, such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness and placed on a positively charged glass slide. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

OptiView Detection system is generally more sensitive than *ultraView* Detection system. The user must validate results obtained with this reagent and detection systems.

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 specificity, sensitivity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-CD3 (2GV6) antibody was determined by testing FFPE normal tissues.

Tissue*	# positive / total cases	Tissue*	# positive / total cases
Adrenal gland	0/3	Nerve, peripheral	0/3
Appendix	0/1	Ovary	0/3
Bladder	0/3	Pancreas	0/3
Breast	0/3	Parathyroid gland	0/3
Cerebellum	0/3	Pharynx, oral cavity	0/3
Cerebrum	0/3	Prostate	0/3
Cervix	0/3	Salivary gland	0/3
Colon	0/3	Skeletal muscle	0/3
Endometrium	0/3	Skin	0/3
Esophagus	0/3	Small intestine	0/3
Heart	0/3	Soft tissue	0/2
Hypophysis (Pituitary)	0/3	Spleen	6/6
Kidney	0/3	Stomach	0/3
Liver	0/3	Testis	0/3
Lung	0/3	Thymus	3/3
Lymph node	9/9	Thyroid	0/3
Mesothelium	0/3	Tonsil	11/11
Myeloid (Bone marrow)	3/3		

* T lymphocytes staining in all non-neoplastic cases.

Table 5. Sensitivity/Specificity of CONFIRM anti-CD3 (2GV6) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Adenoma (adrenal gland)	0/1
Carcinoid Tumor (appendix)	0/1
Squamous Cell Carcinoma (bladder)	0/1
Urothelial Cell Carcinoma (bladder)	0/1
Ductal Carcinoma In Situ (DCIS) (breast)	0/1
Invasive Ductal Carcinoma (breast)	0/1
Invasive Lobular Carcinoma (breast)	0/1
Oligodendroglioma (cerebellum)	0/1
Ependymoma (cerebrum)	0/1

Pathology	# positive / total cases
Glioblastoma (cerebrum)	0/1
Meningioma (cerebrum)	0/1
Squamous Cell Carcinoma (cervix)	0/1
Adenocarcinoma (colon)	0/1
Adenosquamous Carcinoma (colon)	0/1
Adenocarcinoma (endocervical)	0/1
Adenocarcinoma (esophagus)	0/1
Squamous Cell Carcinoma (esophagus)	0/1
Plasmacytoma (extramedullary)	0/1
Adenocarcinoma (head, neck)	0/1
Squamous Cell Carcinoma (head, neck)	0/1
Papillary Renal Adenoma (kidney)	0/1
Renal Clear Cell Carcinoma (kidney)	0/1
Cholangiocarcinoma (liver)	0/1
Hepatocellular Carcinoma (liver)	0/1
NSCLC Adenocarcinoma (lung)	0/1
NSCLC Squamous Cell Carcinoma (lung)	0/1
Small Cell Lung Carcinoma (lung)	0/1
Diffuse large B-cell lymphoma (DLBCL)	0/34
Follicular lymphoma	0/2
MALT B-cell lymphoma	0/8
Non-Hodgkin B cell lymphoma, NOS	4/40
Mantle cell lymphoma	0/1
Hodgkin's Lymphoma	0/20
Non-Hodgkin lymphoma, NOS	3/21
Anaplastic Large Cell Lymphoma	7/15
Angioimmunoblastic T cell lymphoma	12/12
Lymphoma, null type	1/1
Natural Killer/T-cell lymphoma, nasal type	4/5
Natural Killer/T-cell lymphoma, NOS	1/1
Peripheral T cell lymphoma, enteropathy-associated	5/6
Peripheral T cell lymphoma, Lennert lymphoma	2/3
Peripheral T cell lymphoma, mycosis fungoides	1/1
Peripheral T cell lymphoma, NOS	31/33
Mesothelioma (mesothelium)	0/1
Pleural Solitary Fibrous Tumor (mesothelium)	0/1
Alveolar Rhabdomyosarcoma (muscle)	0/1

Pathology	# positive / total cases
Myxoma (muscle)	0/1
Granulosa Cell Tumor (ovary)	0/1
Serous Carcinoma (ovary)	0/1
Teratoma (ovary)	0/1
Ductal Adenocarcinoma (pancreas)	0/1
Islet Cell Tumor (Neuroendocrine Tumor) (pancreas)	0/1
Neurofibrosarcoma (peripheral nerve)	0/1
Schwannoma (peripheral nerve)	0/1
Adenocarcinoma (prostate)	0/2
Pleomorphic Adenoma (salivary gland)	0/1
Warthin's Tumor (salivary gland)	1/1
Basal Cell Carcinoma (skin)	0/1
Invasive Melanoma (skin)	0/1
Squamous Cell Carcinoma (skin)	0/1
Adenocarcinoma (small intestine)	0/1
Gastrointestinal Stromal Tumor (GIST) (small intestine)	0/1
Angiosarcoma (soft tissue)	0/1
Liposarcoma (soft tissue)	0/1
Adenocarcinoma (stomach)	0/1
GIST (stomach)	0/1
Embryonal Carcinoma (testis)	0/1
Seminoma (testis)	0/1
Follicular Carcinoma (thyroid)	0/1
Papillary Carcinoma (thyroid)	0/1
Clear Cell Carcinoma (uterus)	0/1
Endometroid Carcinoma (uterus)	0/1
Leiomyoma (uterus)	0/1
Leiomyosarcoma (uterus)	0/1

Precision

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

- Between lot intermediate precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument intermediate precision on the BenchMark GX, BenchMark XT, and BenchMark ULTRA instruments.
- Between platform intermediate precision between the BenchMark GX, BenchMark XT, and BenchMark ULTRA instruments.

All studies met their acceptance criteria.

REFERENCES

1. Matter M, Schwarz E, Marafioti T, et al. Immunohistochemical detection of CD3 in T-cell lymphomas: superior sensitivity of rabbit monoclonal 2GV6 antibody compared to mouse monoclonal F7-2-38 antibody. *J Histotechnol.* 2014;37(1),21-25.
2. Lanier LL, Chang C, Spits H, Phillips JH. Expression of cytoplasmic CD3 epsilon proteins in activated human adult natural killer (NK) cells and CD3 gamma, delta, epsilon complexes in fetal NK cells. Implications for the relationship of NK and T lymphocytes. *J Immunol.* 1992;149(6),1876-80.
3. Carson F, Hladik C. *Histotechnology: A Self Instructional Text*, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
4. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
5. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
6. Roche PC, Hsi ED. *Immunohistochemistry-Principles and Advances*. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.

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.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see www.ventana.com for definition of symbols used):

GTIN Global Trade Item Number

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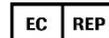
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