

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

REF 790-4341
05278422001

IVD 50

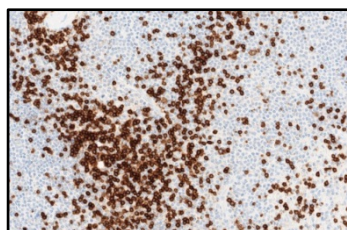


Figure 1. CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody staining of tonsil using OptiView DAB IHC Detection Kit.

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; Ord. Code 06396500001) or *ultraView* Universal DAB Detection Kit (Cat. No. 760-500; Ord. Code 05269806001). Refer to the OptiView DAB IHC Detection Kit or *ultraView* Universal DAB Detection Kit package insert for further information.

REAGENT 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 cell culture supernatant.

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.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

STORAGE

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 tissues are suitable for use with this primary antibody when used with VENTANA detection kits and VENTANA BenchMark IHC/ISH series of automated 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

- For *in vitro* diagnostic (IVD) use.
- For professional use only.
- 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.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as it may cause incorrect results.
- Consult local and/or state authorities with regard to recommended method of disposal.
- For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark IHC/ISH series of automated instruments in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 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 instruments' 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) antibody with OptiView DAB IHC Detection Kit on BenchMark ULTRA, BenchMark XT and BenchMark GX instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	BenchMark ULTRA instrument Cell Conditioning 1, 40 min BenchMark XT instrument Cell Conditioning 1, 40 min BenchMark GX instrument Cell Conditioning 1, 40 min
Enzyme (Protease)	None selected
Pre-primary peroxidase inhibition	Selected
Antibody (Primary)	BenchMark ULTRA instrument 20 minutes, 36°C

Procedure Type	Method
	BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 16 minutes, 37°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for CONFIRM anti-CD3 (2GV6) antibody with *ultraView* Universal DAB Detection Kit on BenchMark ULTRA, BenchMark XT and BenchMark GX instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	BenchMark ULTRA instrument Cell Conditioning 1, Mild BenchMark XT instrument Cell Conditioning 1, Mild BenchMark GX instrument Cell Conditioning 1, Mild
Enzyme (Protease)	None selected
Antibody (Primary)	BenchMark ULTRA instrument 20 minutes, 36°C BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 16 minutes, 37°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".⁴

POSITIVE TISSUE CONTROL

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

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, reproducibility and repeatability were conducted and the results are listed in Table 3, Table 4 and the Repeatability section.

Specificity

Table 3. Specificity of CONFIRM anti-CD3 (2GV6) antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	3/3*
Cerebellum	0/3	Myeloid (bone marrow)	1/3*
Adrenal gland	0/3*	Lung	0/3*
Ovary	0/3*	Heart	0/3
Pancreas	0/3*	Esophagus	0/3*
Parathyroid gland	0/3*	Stomach	0/3*
Hypophysis	0/3*	Small intestine	0/3*
Testis	0/3*	Colon	0/3*
Thyroid	0/3*	Liver	0/3
Breast	0/2*	Salivary gland	0/3*
Spleen	4/4*	Kidney	0/3*
Tonsil	5/5*	Prostate	0/3*
Endometrium	0/3*	Cervix	0/3*
Skeletal muscle	0/3	Skin	0/3*
Nerve (sparse)	0/3*	Mesothelium of lung	0/1
Bladder	0/3*	Lymph node	5/5*

* T lymphocytes staining

Sensitivity

Table 4. Sensitivity of CONFIRM anti-CD3 (2GV6) antibody was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Ovarian serous papillary adenocarcinoma	0/1
Ovarian adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/2
Thyroid medullary carcinoma	0/1
Thyroid papillary carcinoma	0/1
Breast intraductal carcinoma	0/2
Breast invasive ductal carcinoma	0/1
Lung small cell undifferentiated carcinoma	0/1

Pathology	# positive / total cases
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Neuroendocrine carcinoma (esophagus)	0/1
Esophageal adenocarcinoma	0/1
Gastric signet ring cell carcinoma	0/1
Gastrointestinal adenocarcinoma	0/3
GIST	0/3
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	0/2
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Anal malignant melanoma	0/1
Basal cell carcinoma	0/1
Squamous cell carcinoma (skin)	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Malignant mesothelioma	0/1
Hodgkin lymphoma	0/3
Urothelial carcinoma (bladder)	0/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
B-cell lymphoma	1/39
Anaplastic large cell lymphoma	6/8
T-cell lymphoma	60/62

Repeatability

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

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

All studies met their acceptance criteria.

REFERENCES

1. Matter M, Schwarz E, Marafioti T, Rechsteiner MP, Moch H. 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. Roche PC, Hsi ED. *Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology*, 6th edition. (NR Rose Ed.) ASM Press, 2002.

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