CONFIRM anti-Topoisomerase IIα (JS5B4) Rabbit Monoclonal Primary Antibody

REF 790-4371

05479339001

IVD Σ 50

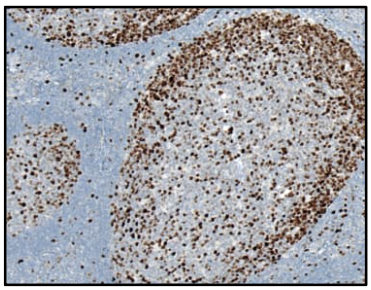

Figure 1. CONFIRM anti-Topoisomerase IIα (JS5B4) antibody staining of tonsil.

INTENDED USE

CONFIRM anti-Topoisomerase IIα (JS5B4) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of topoisomerase IIα by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

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-Topoisomerase IIα (JS5B4) Rabbit Monoclonal Primary Antibody (CONFIRM anti-Topoisomerase IIα (JS5B4) antibody) recognizes DNA topoisomerase IIα. Topoisomerases are nuclear enzymes that alter the topological state of DNA through temporary cleavage and ligation.[1,2] These enzymes create transient breaks in the sugar-phosphate backbone of the DNA double helix and control DNA supercoiling.[2,3] During this process, topoisomerases maintain genomic integrity by forming covalent bonds between active site tyrosine residues and the terminal DNA phosphates that are generated during the cleavage reaction.[4,5] This covalent linkage is the hallmark characteristic of all DNA topoisomerases.[5]

There are two types of topoisomerases, type I and type II.[1,3] Type I enzymes produce single-stranded DNA breaks while type II enzymes produce double-stranded DNA breaks.[1,3] Type I topoisomerases are required in all DNA processes that involve tracking systems and play important roles in maintaining genomic integrity.[2] Type II topoisomerases play critical roles in numerous DNA processes and are required for recombination, separation of daughter chromosomes and proper chromosome structure, condensation, and decondensation.[2,6]

Vertebrate species express two isoforms of type II topoisomerases, topoisomerase IIα and topoisomerase IIβ.[1,2,3] Topoisomerase IIα is essential for the survival of proliferating cells and enzyme concentrations are significantly upregulated during periods of cell proliferation.[1,6,7] Topoisomerase IIα protein levels fluctuate during the cell cycle phases with decreased expression occurring in G1 phase and peak expression occurring in G2/M phase.[6,8,9] Whereas, topoisomerase IIβ is present in non-dividing cells and developing neuronal cells, the cellular function of topoisomerase IIβ has not been fully elucidated.[1,6,7,10]

The detection of topoisomerase IIα by immunohistochemistry (IHC) with the CONFIRM anti-Topoisomerase IIα (JS5B4) antibody may be used to aid in the identification of cell proliferation in normal and neoplastic tissue. It may be used as part of a panel of IHC studies. The staining pattern is nuclear.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Topoisomerase IIα (JS5B4) antibody binds to the topoisomerase IIα antigen in formalin-fixed, paraffin-embedded (FFPE) tissues. This antibody can be visualized using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001), or OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the respective method sheets for further information.

MATERIAL PROVIDED

CONFIRM anti-Topoisomerase IIα (JS5B4) antibody contains sufficient reagent for staining 50 slides.

One 5 mL dispenser of CONFIRM anti-Topoisomerase IIα (JS5B4) antibody contains approximately 1.5 µg 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.

Specific antibody concentration is approximately 0.3 µg/mL. There is no known non-specific antibody reactivity observed in this product.

There is a trace of (~2%) fetal bovine serum of U.S. origin from the stock solution.

CONFIRM anti-Topoisomerase IIα (JS5B4) antibody is a recombinant rabbit monoclonal antibody produced as 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 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. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
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 2-8ºC. Do not freeze.

To ensure proper reagent delivery and 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 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.[11] 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. Do not use beyond the specified number of tests.

4. 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.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. 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.[12,13]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. 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.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	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 reaction mass of 5-chloro-2-methyl-2H-isothiazol-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 the tables below 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 User Guide. 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 790-4371.

Table 2. Recommended staining protocol for CONFIRM anti-Topoisomerase IIα (JS5B4) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	CC1, Standard	ULTRA CC1, 64 minutes, 95 °C (Standard)
Antibody (Primary)	24 minutes, 37 °C	24 minutes, 37 °C	24 minutes, 36 °C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for CONFIRM anti-Topoisomerase IIα (JS5B4) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes	CC1, 64 minutes	ULTRA CC1, 64 minutes, 100 °C
Pre Primary Peroxidase Inhibit.	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37 °C	16 minutes, 37 °C	16 minutes, 36 °C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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."[14]

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 normal tonsil or spleen.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Topoisomerase IIα (JS5B4) antibody is nuclear.

SPECIFIC LIMITATIONS

This antibody may demonstrate weak cytoplasmic staining in striated muscle, most notably in normal cardiac muscle and skeletal muscle. A punctate staining pattern has been observed in the cell cytoplasm in rare instances in breast carcinoma, lung carcinoma, and ovarian carcinoma.

OptiView detection system is generally more sensitive than *ultra*View Universal DAB Detection Kit. The user must validate the 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 sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-Topoisomerase IIα (JS5B4) antibody was determined by testing FFPE tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	2/3	Small intestine	3/3
Cerebellum	2/3	Colon[c]	6/6
Adrenal gland	3/3	Liver	2/3
Ovary	3/3	Salivary gland	3/3
Pancreas	3/3	Pharynx, oral cavity	3/3
Pituitary gland	3/3	Kidney	3/3
Testis	3/3	Prostate	3/3
Thyroid	2/3	Bladder	2/3
Breast[a]	6/6	Parathyroid gland	2/3
Spleen	3/3	Endometrium	3/3
Tonsil	5/5	Cervix	3/3
Thymus	3/3	Skeletal muscle	1/3
Bone marrow	1/3	Skin	3/3
Lung[b]	4/7	Nerve	1/3
Heart	1/3	Mesothelium	3/3
Esophagus	3/3	Soft Tissue	0/2
Stomach	3/3		

[a] Tissues evaluated include breast with fibrocystic changes.

[b] Tissues evaluated include Tuberculosis (TB granuloma).

[c] Tissues evaluated include congenital megacolon.

Table 5. Sensitivity/Specificity of CONFIRM anti-Topoisomerase IIα (JS5B4) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebellum)	1/1
Squamous cell carcinoma (Head and neck)	1/1
Granulosa cell tumor (Ovary)	1/1
Serous carcinoma (Ovary)	1/1
Teratoma (Ovary)	1/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	1/1
Embryonal carcinoma (Testis)	1/1
Papillary carcinoma (Thyroid)	1/1
Follicular carcinoma (Thyroid)	1/1
Adrenal cortical adenoma (Adrenal gland)	0/1
Ductal carcinoma in situ (Breast)	6/6
Invasive lobular carcinoma (Breast)	7/7
Invasive ductal carcinoma (Breast)	72/72
Mucinous adenocarcinoma (Breast)	1/1
Small cell carcinoma (Lung)	4/4
Squamous cell carcinoma (Lung)	48/49
Adenocarcinoma (Lung)	17/17
Papillary adenocarcinoma (Lung)	1/1
Clear cell carcinoma (Lung)	1/1
Carcinoid (Lung)	1/1
Bronchioloalveolar carcinoma (Lung)	11/11
Adenosquamous carcinoma (Lung)	9/9
Undifferentiated carcinoma (Lung)	4/4
Undifferentiated carcinoma, large cell (Lung)	1/1
Warthin's tumor (Salivary gland)	1/1
Pleomorphic adenoma (Salivary gland)	1/1
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Gastrointestinal stromal tumor (GIST) (Stomach)	1/1
Adenocarcinoma (Small intestine)	1/1
GIST (Small intestine)	1/1
Adenocarcinoma (Colon)	84/84
Adenosquamous carcinoma (Colon)	1/1
Mucinous adenocarcinoma (Colon)	7/7
Papillary adenocarcinoma (Colon)	3/3

Pathology	# positive / total cases
Adenoma (Colon)	1/1
Tubular adenoma (Colon)	1/1
Carcinoid tumor (Appendix)	1/1
Cholangiocarcinoma (Liver)	1/1
Hepatocellular carcinoma (Liver)	1/1
Papillary renal adenoma (Kidney)	1/1
Renal cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Leiomyosarcoma (Uterus)	1/1
Adenocarcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	1/1
Adenocarcinoma (Cervix)	1/1
Alveolar rhabdomyosarcoma (Striated muscle)	1/1
Myxoma (Muscle)	0/1
Melanoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Malignant peripheral nerve sheath tumor (Peripheral nerve)	1/1
Mesothelioma (Mesothelium)	1/1
Pleural solitary fibrous tumor (Mesothelium)	1/1
Anaplastic Large Cell Lymphoma (Lymph node)	1/1
Hodgkin Lymphoma (Lymph node)	1/1
Urothelial carcinoma (Bladder)	1/1
Angiosarcoma (Soft tissue)	1/1
Liposarcoma (Soft tissue)	1/1

Precision

Precision studies for CONFIRM anti-Topoisomerase IIα (JS5B4) antibody were completed to demonstrate:

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

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

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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.

The summary of safety and performance can be found here:
https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
E	Updates to Warnings and Precautions section. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

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