CONFIRM anti-p53 (DO-7) Primary Antibody

REF 790-2912

05278074001

IVD Σ 50

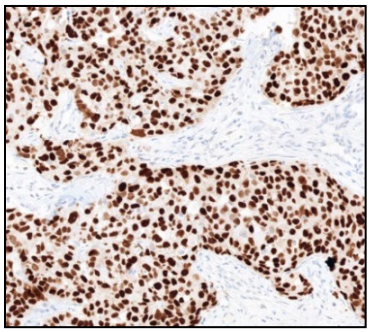

Figure 1. CONFIRM anti-p53 (DO-7) antibody exhibiting a nuclear staining pattern in adenocarcinoma of the colon.

INTENDED USE

CONFIRM anti-p53 (DO-7) Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of p53 protein 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-p53 (DO-7) Primary Antibody (CONFIRM anti-p53 (DO-7) antibody) recognizes tumor protein p53 (TP53) also known as p53. The p53 protein is a 43.7 kDa DNA binding protein comprised of 393 amino acids. It consists of six domains: the transcription activation domain I (TAD 1); the transcription activation domain II (TAD II); the proline-rich region (PRD); the central core domain, which includes the DNA binding domain (DBD); the tetramerization domain (TD), and the C-terminal basic domain (BD).[1] Full-length p53 can reversibly form tetramers via the TD.[2] Tetramer formation of p53 is essential for site-specific DNA binding, posttranslational modifications, and protein-protein interactions.[2]

The p53 protein plays a central role in maintaining genomic integrity by halting the cell cycle upon genomic stress and hindering proliferation of cells with damaged DNA.[2,3,4,5] The p53 protein is maintained at very low levels in unstimulated cells; however, in response to various stimuli such as DNA damage, nutrient deprivation, ribonucleotide depletion, hypoxia, oxidative stress, and hyper-proliferative signals, p53 becomes stabilized and accumulates in the cells.[3] Once activated, p53 mediates changes in gene expression and promotes cell cycle arrest, apoptosis, and DNA repair.[3,6] Therefore, inactivation of p53 can result in proliferation and transformation of compromised cells.[5]

The detection of wild type and mutant p53 protein by immunohistochemistry (IHC) with the CONFIRM anti-p53 (DO-7) antibody may be used to provide ancillary information regarding the accumulation of p53, which is associated with cell cycle dysregulation and loss of tumor suppression. It may be used as part of a panel of IHC studies. The staining pattern is nuclear.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-p53 (DO-7) antibody is a mouse monoclonal antibody produced against a recombinant human wild type p53 protein. CONFIRM anti-p53 (DO-7) antibody binds to the p53 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections and exhibits a nuclear staining pattern. 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 sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-p53 (DO-7) Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-p53 (DO-7) Primary Antibody contains approximately 2.5 µg of a mouse monoclonal antibody.

The antibody is diluted in a phosphate buffered saline containing carrier protein and 0.05% ProClin 300 as a preservative.

Specific antibody concentration is approximately 0.5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-p53 (DO-7) antibody is a mouse monoclonal antibody produced as protein A purified supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principles of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Interpretation of Results, Limitations and Troubleshooting.

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. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *ultra*View 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. General purpose laboratory equipment
15. 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 FFPE tissues are suitable for use with this primary antibody when used with a VENTANA detection kit and a VENTANA BenchMark IHC/ISH instrument. The recommended tissue fixative is 10% neutral buffered formalin.[7] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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. Ask your Roche representative for more information on 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.[8,9]
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 User Guide, and instructions for use of all necessary components located at dialog.roche.com.
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, 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 Table 2 and Table 3 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 package insert 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 790-2912.

Table 2. Recommended staining protocol for CONFIRM anti-p53 (DO-7) 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 (Standard), 95 °C
Antibody (Primary)	24 minutes, 37 °C	24 minutes, 37 °C	28 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-p53 (DO-7) 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, 32 minutes	CC1, 32 minutes	ULTRA CC1, 32 minutes, 100 °C
Antibody (Primary)	16 minutes, 37 °C	16 minutes, 37 °C	16 minutes, 36 °C
Pre-Primary Peroxidase Inhibitor	Selected		
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
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".[10]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-p53 (DO-7) antibody, a second slide should be stained with the appropriate negative control reagent.

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.

An example of a positive control tissue is colonic adenocarcinoma, which should demonstrate strong nuclear staining in the majority of neoplastic cells.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-p53 (DO-7) Primary Antibody is nuclear.

SPECIFIC LIMITATIONS

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

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-p53 (DO-7) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Stomach	5/5
Cerebellum	0/3	Small intestine	2/3
Adrenal gland	1/3	Colon[b]	4/7
Ovary	3/5	Appendix	2/2
Pancreas	3/6	Liver	0/6
Lymph node	1/1	Salivary gland	3/3
Parathyroid gland	2/3	Pharynx, oral cavity	2/3
Pituitary gland	1/3	Kidney	2/3
Testis	3/3	Prostate	3/3
Thyroid	2/3	Bladder	3/3
Breast[a]	3/8	Endometrium	3/3
Spleen	0/3	Cervix	3/3
Tonsil	4/4	Skeletal muscle	0/3
Thymus	6/6	Skin	3/3
Bone marrow	0/3	Nerve	1/4
Lung	1/3	Mesothelium	3/3
Heart	0/3	Soft Tissue	1/2
Esophagus	3/3		

[a] Tissues evaluated included normal breast and breast with fibrocystic changes.

[b] Tissues evaluated included normal colon and congenital megacolon.

Table 5. Sensitivity/Specificity of CONFIRM anti-p53 (DO-7) Primary 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
Adenocarcinoma (Head and neck)	1/1
Squamous cell carcinoma (Head and neck)	1/1
Serous adenocarcinoma (Ovary)	37/49
Mucinous adenocarcinoma (Ovary)	11/13
Granulosa cell tumor (Ovary)	1/1
Teratoma (Ovary)	0/1
Serous cystadenoma (Ovary)	1/1
Mucinous cystadenoma (Ovary)	0/1
Borderline mixed cystadenoma (Ovary)	1/1
Endometrioid carcinoma (Ovary)	25/28
Clear cell carcinoma (Ovary)	1/2
Neuroendocrine neoplasm (Pancreas)	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
Ductal carcinoma in situ (Breast)	4/7
Invasive ductal carcinoma (Breast)	58/75
Invasive lobular carcinoma (Breast)	4/11
Adrenal cortical adenoma (Adrenal gland)	1/1
Small cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Pleomorphic adenoma (Salivary gland)	1/1
Warthin tumor (Salivary gland)	1/1
Adenocarcinoma (Esophagus)	1/1
Adenocarcinoma (Stomach)	1/1
Gastrointestinal stromal tumor (GIST) (Stomach)	1/1
Adenocarcinoma (Small intestine)	0/1
GIST (Small intestine)	1/1
Adenocarcinoma (Colon)	67/80
Adenosquamous carcinoma (Colon)	1/1
Mucinous adenocarcinoma (Colon)	9/10
Papillary adenocarcinoma (Colon)	2/3
Adenoma, NOS (Colon)	0/1
Tubular adenoma (Colon)	2/2
Well differentiated neuroendocrine tumor (Appendix)	1/1
Hepatocellular carcinoma (Liver)	0/1

Pathology	# positive / total cases
Cholangiocarcinoma (Liver)	1/1
Papillary adenoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Clear cell carcinoma (Uterus)	1/1
Endometrioid carcinoma (Uterus)	1/1
Leiomyoma (Uterus)	0/1
Leiomyosarcoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	1/1
Adenocarcinoma (Endocervix)	0/1
Alveolar rhabdomyosarcoma (Muscle)	1/1
Myxoma (Muscle)	1/1
Basal cell carcinoma (Skin)	1/1
Melanoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Malignant peripheral nerve sheath tumor (Peripheral nerve)	1/1
Schwannoma (Peripheral nerve)	1/1
Hodgkin lymphoma (Lymph node)	1/1
Squamous cell carcinoma (Bladder)	1/1
Plasmacytoma (Extramedullary)	0/1
Mesothelioma (Mesothelium)	1/1
Solitary fibrous tumor (Pleura)	1/1
Angiosarcoma (Soft tissue)	0/1
Liposarcoma (Soft tissue)	1/1

Precision

Precision studies for CONFIRM anti-p53 (DO-7) 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 a BenchMark XT, BenchMark GX, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, BenchMark XT, and 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.

REFERENCES

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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.

Symbols

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

GTIN	Global Trade Item Number
UDI	Unique Device Identification

REVISION HISTORY

Rev	Updates
E	Updates to Intended Use, Summary and Explanation, Principle of the Procedure, Material Provided, Materials Required But Not Provided, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Specific Limitations, Analytical Performance, References, Symbols, and Intellectual Property sections. Added BenchMark GX, ULTRA, and ULTRA PLUS instruments. Added recommended protocols for OptiView DAB IHC Detection Kit and *ultra*View Universal DAB Detection Kit. Removed recommended protocols for *i*VIEW DAB detection kit.

INTELLECTUAL PROPERTY

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