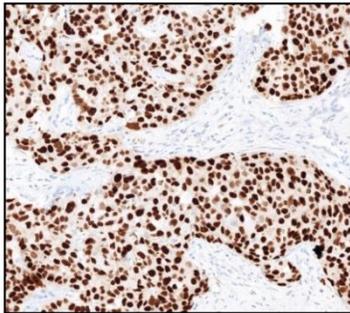


## CONFIRM anti-p53 (DO-7) Primary Antibody

**REF** 800-2912  
05278775001

**IVD**  50



**Figure 1. CONFIRM anti-p53 (DO-7) Primary Antibody exhibiting a nuclear staining pattern in colon adenocarcinoma tissue.**

### INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-p53 (DO-7) Primary Antibody is a mouse monoclonal antibody (IgG1, kappa) directed against human p53. The antibody is intended for laboratory use to qualitatively identify by light microscopy wild type and mutant p53 in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer.

The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be

made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

**CAUTION:** U.S. Federal law restricts this device to sale by or on the order of a physician. This antibody is intended for *in vitro* diagnostic (IVD) use.

### SUMMARY AND EXPLANATION

The wild type p53 molecule has a role in transcription, in cell cycle control and in many other metabolic functions. It is also a tumor suppressor in that it halts abnormal growth in normal cells; however, a mutation in one of its 393 amino acids can eliminate the surveillance capability of the protein and allow expression of malignant phenotype. Approximately 50% of all human cancers contain a mutation in p53. The wild type molecule has a short half-life and is present in low concentrations; therefore, it cannot usually be detected by immunohistochemistry (IHC). Many of the mutations in p53 increase its half-life and cause intra-nuclear accumulation of the mutant protein, making it detectable by IHC.<sup>1,2</sup> Thus in many malignancies of the colon, bladder, ovary, breast and mesothelium, p53 protein can be readily detected in the malignant cells by IHC, and immunostaining closely correlates with the presence of a mutation.<sup>1-5</sup> The staining pattern with the anti-p53 monoclonal antibody DO-7 is exclusively nuclear and may be present in a heterogeneous pattern.<sup>1-3</sup>

### PRINCIPLE OF THE PROCEDURE

VENTANA CONFIRM anti-p53 (DO-7) Primary Antibody is a mouse monoclonal antibody produced against a recombinant human wild type p53 protein. CONFIRM anti-p53 (DO-7) Primary Antibody binds to the p53 protein in paraffin-embedded tissue sections and exhibits a nuclear staining pattern. This antibody can be visualized using VUEW DAB Detection Kit (Cat. No. 760-091 / 05266157001). Refer to the package insert for further information.

### REAGENT 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.

Total protein concentration of the reagent is approximately 3 mg/mL. 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) Primary Antibody is a mouse monoclonal antibody produced as protein A purified supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: Principles of the Procedure, Materials and Reagents Needed but Not Provided, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Interpretation of Results, General 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 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 a VENTANA detection kit and a VENTANA BenchMark IHC/ISH instrument. The recommended tissue fixative is 10% neutral buffered formalin.<sup>6</sup> 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. 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.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at [www.ventana.com](http://www.ventana.com).

### 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 1 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 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-p53 (DO-7) Primary Antibody with /VIEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 Standard CC1
Enzyme (protease)	None required
Antibody (Primary)	16 minutes, 37°C
A/B Block (Biotin blocking)	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".<sup>7</sup>

#### POSITIVE TISSUE CONTROL

The recommended 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

The user must validate the results obtained with this reagent and detection system.

#### PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity and repeatability were conducted and the results are listed in Table 2 and Table 3 and in the Repeatability section.

#### Specificity

**Table 2.** Specificity of CONFIRM anti-p53 (DO-7) Primary Antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Mesothelium	0/3
Pancreas	0/3	Heart	0/3
Parathyroid gland	0/3	Esophagus	0/3
Hypophysis (pituitary)	0/3	Stomach	1/4 <sup>a</sup>
Testis	0/3	Small intestine	0/3
Thyroid	0/4	Colon	0/5 <sup>d</sup>
Breast	6/32 <sup>b</sup>	Liver	0/3
Spleen	1/3 <sup>a</sup>	Salivary gland	0/3
Tonsil	5/8 <sup>b,c</sup>	Kidney	0/3
Endometrium	0/3	Prostate	0/3
Skeletal muscle	0/4	Cervix	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Peripheral nerve	0/3	Skin	0/3
Bladder	0/3		

<sup>a</sup> All positive cases show ≤1% cells staining

<sup>b</sup> All positive cases show ≤5% cells staining

<sup>c</sup> Few epithelial cells and lymphoid cells staining

<sup>d</sup> Rare lymphocytes (<1%) staining

#### Sensitivity

**Table 3.** Sensitivity of CONFIRM anti-p53 (DO-7) Primary Antibody was determined by testing a variety of formalin-fixed, paraffin-embedded pathologic tissues.

Pathology	# positive / total cases
Glioblastoma	1/1 <sup>a</sup>
Meningioma	0/1
Anaplastic ependymoma	0/1
Oligodendroglioma	0/1
Ovarian serous adenocarcinoma	1/1
Ovarian adenocarcinoma	1/1 <sup>a</sup>
Pancreatic endocrine neoplasm	1/1 <sup>a</sup>
Pancreatic adenocarcinoma	1/1 <sup>b</sup>
Seminoma	1/1
Testicular embryonal carcinoma	1/1 <sup>a</sup>
Thyroid medullary carcinoma	1/1 <sup>a</sup>
Thyroid papillary carcinoma	1/1 <sup>a</sup>
Lung small cell carcinoma	1/1
Lung squamous cell carcinoma	1/1
Lung adenocarcinoma	0/1
Esophageal squamous cell carcinoma	1/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	0/1
Small bowel adenocarcinoma	0/1
Gastrointestinal stromal tumor (GIST)	2/3 <sup>a</sup>
Congenital megacolon	0/1
Colonic adenoma	0/2
Colorectal adenocarcinoma	77/90
Colonic mucinous adenocarcinoma	7/8
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/2 <sup>a</sup>
Urothelial carcinoma	2/2
Uterine leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1

Pathology	# positive / total cases
Cervical squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	1/1 <sup>a</sup>
Melanoma	0/1
Basal cell carcinoma	0/1
Skin squamous cell carcinoma	0/1
Neurofibroma	0/1
Neuroblastoma	0/1
Mesothelioma	1/1
Splenic B-cell lymphoma, NOS	1/1 <sup>a</sup>
Lymph node B-cell lymphoma, NOS	0/2
Hodgkin's lymphoma	0/1
Anaplastic large cell lymphoma	0/1
Leiomyosarcoma	1/2 <sup>a</sup>
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Fibrocystic changes	1/10 <sup>a</sup>
Fibroadenoma	2/10 <sup>a</sup>
Atypical hyperplasia	3/9
Ductal carcinoma in situ (DCIS)	10/15
Invasive ductal carcinoma	74/110
Breast mucinous adenocarcinoma	1/1
Invasive lobular carcinoma	4/11

<sup>a</sup> All positive cases show ≤1% cells staining

<sup>b</sup> All positive cases show ≤5% cells staining

### Repeatability

Repeatability studies for CONFIRM anti-p53 (DO-7) Primary Antibody were completed to demonstrate:

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

All studies met their acceptance criteria.

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