

## Anti-p53 (Bp53-11) Primary Antibody

REF

760-2542

05267102001

IVD

Σ 50

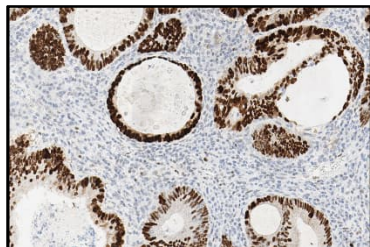


Figure 1. Anti-p53 (Bp53-11) antibody staining of adenocarcinoma of the colon.

diagnostic (IVD) use.

### SUMMARY AND EXPLANATION

Anti-p53 (Bp53-11) Primary 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 I); 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).<sup>1</sup> Full-length p53 can reversibly form tetramers via the TD.<sup>2</sup> Tetramer formation of p53 is essential for site-specific DNA binding, posttranslational modifications, and protein-protein interactions.<sup>2</sup>

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.<sup>2,3,4,5</sup> 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.<sup>3</sup> Once activated, p53 mediates changes in gene expression and promotes cell cycle arrest, apoptosis, and DNA repair.<sup>3,6</sup> Therefore, inactivation of p53 can result in proliferation and transformation of compromised cells.<sup>5</sup>

The detection of wild type and mutant p53 protein by immunohistochemistry (IHC) with the Anti-p53 (Bp53-11) Primary 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

Anti-p53 (Bp53-11) Primary Antibody is a mouse monoclonal antibody directed against the p53 protein. Anti-p53 (Bp53-11) Primary Antibody binds to the p53 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001) or *OptiView* DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) on a BenchMark IHC/ISH instrument. Refer to the respective method sheet for further information.

### MATERIAL PROVIDED

Anti-p53 (Bp53-11) Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of Anti-p53 (Bp53-11) Primary Antibody contains approximately 12.5 µg of a mouse monoclonal antibody directed against an epitope present on the human p53 antibody.

The antibody is diluted in phosphate buffered saline containing carrier protein and preservative.

Specific antibody concentration is approximately 2.5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-p53 (Bp53-11) Primary Antibody is a mouse 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. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
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. 760-2208 / 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 VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.<sup>7</sup> 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.

Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

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.<sup>8,9</sup>

- 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.
- 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](http://navifyportal.roche.com).
- Consult local and/or state authorities with regard to recommended method of disposal.
- Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
- 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
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing mist or vapours.
	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 the tables 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 760-2542.

Table 2. Recommended staining protocol for Anti-p53 (Bp53-11) Primary Antibody with ultraView Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS <sup>a</sup>
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA CC1, 36 minutes (Mild), 95 °C
Antibody (Primary)	24 minutes, 37 °C	24 minutes, 37 °C	32 minutes, 36 °C
Counterstain	Hematoxylin II, 4 minutes		

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS <sup>a</sup>
Post Counterstain	Bluing, 4 minutes		

<sup>a</sup> Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for Anti-p53 (Bp53-11) Primary Antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS <sup>a</sup>
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 32 minutes	CC1, 32 minutes	ULTRA CC1, 32 minutes, 100 °C
Antibody (Primary)	12 minutes, 37 °C	16 minutes, 37 °C	12 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		

<sup>a</sup> 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."<sup>10</sup>

## NEGATIVE REAGENT CONTROL

In addition to staining with Anti-p53 (Bp53-11) Primary Antibody, a second slide should be stained with the appropriate negative control reagent.

## POSITIVE TISSUE CONTROL

A positive tissue control must be included with each staining run. 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 cells 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 the 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 for this antibody is colonic adenocarcinoma, which should demonstrate nuclear staining in the majority of neoplastic cells.

## STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for Anti-p53 (Bp53-11) Primary Antibody is nuclear.

## SPECIFIC LIMITATIONS

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 Anti-p53 (Bp53-11) Primary Antibody was determined by testing FFPE normal tissues.

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

<sup>a</sup> Tissues evaluated included normal breast and breast with fibrocystic changes.

<sup>b</sup> Tissues evaluated included normal colon and congenital megacolon.

Table 5. Sensitivity/Specificity of Anti-p53 (Bp53-11) 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)	32/49
Mucinous adenocarcinoma (Ovary)	11/17

Pathology	# positive / total cases
Granulosa cell tumor (Ovary)	1/1
Teratoma (Ovary)	1/1
Serous cystadenoma (Ovary)	1/1
Mucinous cystadenoma (Ovary)	1/1
Borderline mixed cystadenoma (Ovary)	1/1
Endometrioid carcinoma (Ovary)	24/28
Clear cell carcinoma (Ovary)	2/2
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)	5/8
Invasive ductal carcinoma (Breast)	60/79
Invasive lobular carcinoma (Breast)	5/11
Mucinous adenocarcinoma (Breast)	1/1
Adrenal cortical adenoma (Adrenal gland)	1/1
Pheochromocytoma (Adrenal gland)	1/1
Small cell carcinoma (Lung)	1/1
Adenocarcinoma (Lung)	1/1
Pleomorphic adenoma (Salivary gland)	1/1
Warthin tumor (Salivary gland)	1/1
Adenocarcinoma (Esophagus)	1/1
Squamous cell carcinoma (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)	71/83
Adenosquamous carcinoma (Colon)	1/1
Mucinous adenocarcinoma (Colon)	8/8
Papillary adenocarcinoma (Colon)	3/3
Adenoma, NOS (Colon)	1/1
Tubular adenoma (Colon)	0/1
Well differentiated neuroendocrine tumor (Appendix)	1/1
Hepatocellular carcinoma (Liver)	0/1
Cholangiocarcinoma (Liver)	1/1
Papillary adenoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Clear cell carcinoma (Uterus)	1/1
Endometrioid carcinoma (Uterus)	1/1

Pathology	# positive / total cases
Leiomyoma (Uterus)	0/1
Leiomyosarcoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	1/1
Adenocarcinoma (Endocervix)	1/1
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
Anaplastic large cell lymphoma (Lymph node)	1/1
Hodgkin lymphoma (Lymph node)	1/1
Squamous cell carcinoma (Bladder)	1/1
Plasmacytoma (Extramedullary)	1/1
Mesothelioma (Mesothelium)	1/1
Solitary fibrous tumor (Pleura)	1/1
Angiosarcoma (Soft tissue)	1/1
Liposarcoma (Soft tissue)	1/1

## Precision

Precision studies for Anti-p53 (Bp53-11) Primary 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, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT, BenchMark GX, 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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- Directive 2000/54/EC of the European Parliament and Council of 24 June 2000 on the protection of workers from risks related to exposure to biological agents at work
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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](https://elabdoc.roche.com/symbols) for definition of symbols used):



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. Updated to current template.

## INTELLECTUAL PROPERTY

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