anti-p40 (BC28) Mouse Monoclonal Primary Antibody

REF 790-4950

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IVD Σ 50

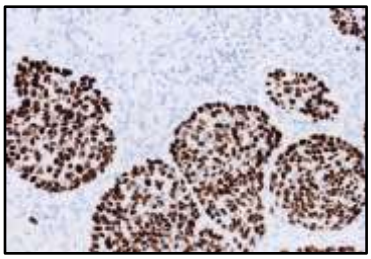

Figure 1. Anti-p40 (BC28) antibody staining of lung squamous cell carcinoma.

INTENDED USE

Anti-p40 (BC28) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of the p40 protein by light microscopy in 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

Anti-p40 (BC28) Mouse Monoclonal Primary Antibody (anti-p40 (BC28) antibody) is a mouse monoclonal antibody raised for the detection of the p40 isoform of the p63 protein. The p63 protein is a member of the p53 family of transcription factors.[1] Five isoforms of p63 (α, β, γ, δ, ε) can be produced due to alternative splicing and an additional 5 isoforms lacking the N-terminal transactivation domain (ΔNp63/p40 α, β, γ, δ, ε) can be produced due to the presence of a second promoter site within the gene.[1,2]

p40 is expressed in the basal or progenitor cells of epithelial tissues and glandular structures including the prostate and bronchi.[3,4] In the prostate, p40 is expressed in the basal cells of almost all normal and benign glands but is not present in the neuroendocrine or luminal secretory cells.[4,5] The presence of basal cells, as marked by the presence of p40 staining, is a feature of normal and benign prostatic processes.[5] The absence of basal cells is a hallmark of prostatic malignancies.[6] The immunohistochemical (IHC)-based detection of p40 in prostatic basal cells, using anti-p40 (BC28) antibody can be used to aid in the differentiation of benign and malignant prostate lesions.

In lung, p40 is expressed in the basal cell compartment.[3] It has been speculated that squamous cell carcinoma (SCCA) of the lung originates from the basal compartment.[7] Malignant squamous differentiation is characteristic of the SCCA subtype of non-small cell lung cancer (NSCLC).[8] Thus, the overexpression of p40 in NSCLC can be an indicator of malignant squamous differentiation.[8,9,10] The adenocarcinoma (ADC) subtype of NSCLC typically does not exhibit p40 expression like SCCA.[8] Thus, detection of p40 using anti-p40 (BC28) antibody may be used as a marker of squamous differentiation to aid in the distinction between pulmonary SCCA and ADC.

In contrast to the anti-p63 antibody (clone 4A4) that recognizes p63 and p40 variants, the anti-p40 (BC28) antibody recognizes only the p40 isoform.[9] Studies indicate that anti-p40 (BC28) antibody is more specific for SCCA than anti-p63 (4A4) antibody.[9,10] Thus, it is recommended to use anti-p40 (BC28) antibody to aid in the distinction between pulmonary SCCA and ADC.[11]

This antibody may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

Anti-p40 (BC28) antibody is a mouse monoclonal antibody produced against a synthetic peptide representing the p40 amino acid sequence from amino acid 5 to 17. Anti-p40 (BC28) antibody binds to p40 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections and exhibits a nuclear staining pattern. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001) in combination with Amplification Kit (Cat. No. 760-080 / 05266114001). Refer to the respective method sheet for further information.

In addition to staining with the anti-p40 (BC28) antibody, a second slide should be stained with the appropriate negative control reagent.

MATERIAL PROVIDED

Anti-p40 (BC28) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-p40 (BC28) antibody contains approximately 2.0 µg of a mouse monoclonal antibody.

The antibody is diluted in Tris-HCl with carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 0.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

The anti-p40 (BC28) antibody is a mouse monoclonal antibody produced as an ascites material.

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. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500/05269806001)
6. Amplification Kit (Cat. No. 760-080/05266114001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
14. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
15. Permanent mounting medium
16. Cover glass
17. Automated coverslipper
18. General purpose laboratory equipment
19. 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.[12] 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.[13,14]
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.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	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, 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 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 790-4950.

Table 2. Recommended staining protocol for anti-p40 (BC28) 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, 100°C	CC1, 32 minutes, 100°C	ULTRA CC1 32 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	16 minutes, 36°C
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.

Table 3. Recommended staining protocol for anti-p40 (BC28) antibody with ultraView 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, Standard
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	16 minutes, 36°C
Amplification	Selected	Selected	Selected
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."[15]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-p40 (BC28) 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.

Examples of positive control tissues for this antibody are basal cells in normal prostate and lung squamous cell carcinoma.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for anti-p40 (BC28) antibody is nuclear.

SPECIFIC LIMITATIONS

The specimen should be fixed within 24 hours of collection with 10% neutral buffered formalin for 12-24 hours. It is not recommended to fix tissues with 95% alcohol and Z-5 fixative.

Anti-p40 (BC28) antibody was found infrequently to exhibit very weak, focal staining in adenocarcinoma (< 5% cells).

OptiView detection system is generally more sensitive than *ultra*View detection system. 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-p40 (BC28) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus [b]	3/3
Cerebellum	0/3	Myeloid (Bone marrow)	0/3
Adrenal gland	0/3	Lung	0/13
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus [b]	1/3
Parathyroid gland	0/3	Stomach	0/3
Hypophysis (Pituitary)	0/3	Small intestine	0/3
Testis	0/3	Colon	0/3
Thyroid	0/3	Liver	0/3
Breast [a]	3/3	Salivary gland [b]	1/3
Spleen	0/3	Kidney	0/3
Tonsil [b]	3/3	Prostate [d]	8/9
Endometrium	0/3	Cervix [b]	1/3
Skeletal muscle	0/3	Skin [b]	3/3
Nerve	0/3	Mesothelium and lung	0/3
Bladder [c]	2/3	Lymph node	0/3

[a] myoepithelial cells; [b] squamous cells; [c] urothelial cells; [d] basal cells

Table 5. Sensitivity/Specificity of anti-p40 (BC28) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous carcinoma (Ovary)	1/1
Carcinoma (Ovary)	1/1
Pancreatic neuroendocrine tumor (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/2
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
Small cell carcinoma (Lung)	0/7
Squamous cell carcinoma (Lung)	74/92
Adenocarcinoma (Lung)	12/145
Large cell carcinoma (Lung)	1/4
Neuroendocrine carcinoma, atypical carcinoid tumor (Lung)	0/5
Adenocarcinoma in situ (Lung)	0/4
Adenosquamous carcinoma (Lung) [a]	2/2
Neuroendocrine carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Signet-ring cell carcinoma	0/1
Adenocarcinoma (Small intestine)	0/1
Stromal sarcoma	0/1
Gastrointestinal stromal tumor (GIST)	0/2
Adenocarcinoma (Colorectal)	0/2
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/57
Prostatic hyperplasia (Prostate)	3/3
Leiomyoma (Uterus)	0/1
Carcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Uterus)	2/2

Pathology	# positive / total cases
Embryonal rhabdomyosarcoma (Striate muscle)	0/1
Melanoma (Anus)	0/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Epithelioid mesothelioma	0/1
B-cell lymphoma, NOS (Lymph node)	0/3
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma (Bladder)	0/2
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma (Peritoneum)	0/1

[a] Squamous component

Precision

Precision studies for anti-p40 (BC28) 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 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.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of anti-p40 (BC28) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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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 dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
C	Updates to Material Provided, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Analytical Performance, and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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