

anti-p40 (BC28) Mouse Monoclonal Primary Antibody

REF 790-4950

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IVD  50

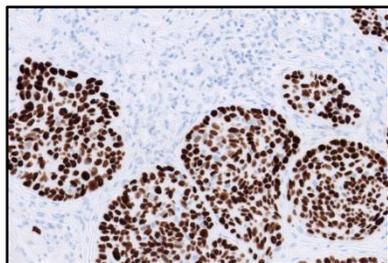


Figure 1. Anti-p40 (BC28) Mouse Monoclonal Primary Antibody staining of lung squamous cell carcinoma using OptiView DAB IHC Detection Kit.

INTENDED USE

Anti-p40 (BC28) Mouse Monoclonal Primary Antibody [anti-p40 (BC28)] is intended for laboratory use in the detection of the p40 protein in formalin-fixed, paraffin-embedded tissue.

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.

Total protein concentration of the reagent is approximately 10 mg/mL. 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) is a mouse monoclonal antibody produced as an ascites material. Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General 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 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 VENTANA detection kits and VENTANA BenchMark ULTRA, BenchMark XT and BenchMark GX automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.⁶ 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. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark ULTRA, BenchMark XT and BenchMark GX automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1 and 2 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.

SUMMARY AND EXPLANATION

The transcription factor p63 has been identified as a p53 homologue. There are at least 6 different isoforms of p63 because of alternative promoter sites and alternative splicing. The two best characterized p63 variants are long TAp63 isoform containing N-terminal transactivation domain and a truncated variant p40 (ΔNp63) lacking the N-terminal domain. These two variants have different functions; TAp63 activates p53 target genes, while p40 (ΔNp63) inhibits transcription activation of p53 genes.¹⁻²

The anti-p40 (BC28) is a mouse monoclonal antibody produced against p40 protein. In contrast to the anti-p63 antibody (clone 4A4) that recognizes both p63 variants, the anti-p40 (BC28) recognizes only the p40 (ΔNp63) isoform.³ Although anti-p63 (4A4) has high sensitivity in lung squamous cell carcinoma, a limitation of this antibody is low specificity due to its reactivity in a substantial proportion of lung adenocarcinomas and other tumor types. Studies indicate that anti-p40 (BC28) is more specific than anti-p63 (4A4) and thus, it is recommended to use anti-p40 (BC28) instead of anti-p63 (4A4) for the diagnosis of pulmonary squamous cell carcinoma.³⁻⁵ In addition, anti-p40 (BC28) was shown to be a valuable marker for the identification of squamous cell carcinoma of head and neck and urothelial carcinomas.³ In the prostate, p40 can be detected in the basal cells of almost all normal and benign glands, but is not present in the neuroendocrine or luminal cells. As basal cells are absent from invasive prostate adenocarcinoma, p40 expression can be used to differentiate between benign and malignant glands.³ Similarly, p40 expression in breast myoepithelial cells may be useful in differentiating between benign and malignant breast lesions.³

PRINCIPLE OF THE PROCEDURE

Anti-p40 (BC28) is a mouse monoclonal antibody produced against synthetic peptide representing the amino acid sequence from amino acid 5 to 17 (PVLEPGDKPRKAT). Anti-p40 (BC28) binds to p40 protein in paraffin-embedded 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 *ultraView* Universal DAB Detection Kit (Cat. No. 760-500/05269806001) in combination with Amplification Kit (Cat. No. 760-080/05266114001). Refer to the OptiView DAB IHC Detection Kit or *ultraView* Universal DAB Detection Kit package inserts and Amplification Kit package insert for further information.

REAGENT PROVIDED

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

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

The antibody is diluted in 0.05M Tris-HCL with 1% carrier protein and ProClin 300, a preservative.

Table 1. Recommended Staining Protocol for anti-p40 (BC28) with OptiView DAB IHC Detection Kit on a BenchMark ULTRA instrument, BenchMark XT instrument and BenchMark GX instrument.

| Procedure Type | Method |
|---------------------------------------|--|
| Deparaffinization | Selected |
| Cell Conditioning (Antigen Unmasking) | Cell Conditioning 1, 32 minutes |
| Enzyme (Protease) | None required |
| Pre-primary peroxidase inhibition | Selected |
| Antibody (Primary) | BenchMark ULTRA instrument 16 minutes, 36°C BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 16 minutes, 37°C |
| OptiView HQ linker | 8 minutes |
| OptiView HRP Multimer | 8 minutes |
| Counterstain Detection kit | Hematoxylin II, 4 minutes |
| Post Counterstain | Bluing, 4 minutes |

Table 2. Recommended Staining Protocol for anti-p40 (BC28) with *ultra*View Universal DAB Detection Kit on a BenchMark ULTRA instrument, BenchMark XT instrument and BenchMark GX instrument.

| Procedure Type | Method |
|---------------------------------------|--|
| Deparaffinization | Selected |
| Cell Conditioning (Antigen Unmasking) | Cell Conditioning 1, Standard |
| Enzyme (Protease) | None required |
| Antibody (Primary) | BenchMark ULTRA instrument 16 minutes, 36°C BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 16 minutes, 37°C |
| Amplification | Selected |
| 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 or cell conditioning based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁷

POSITIVE TISSUE CONTROL

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) 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) was found infrequently to exhibit very weak, focal staining in adenocarcinoma (<5% cells).

This antibody has been optimized for a 16 minute incubation time on a BenchMark ULTRA in combination with OptiView DAB IHC Detection Kit and *ultra*View Universal DAB Detection Kit with Amplification Kit but the user must validate results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 3 and Table 4 and in the Repeatability section.

Specificity

Table 3. Specificity of anti-p40 (BC28) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

| Tissue | # positive / total cases | Tissue | # positive / total cases |
|-------------------|--------------------------|-----------------------|--------------------------|
| Cerebrum | 0/3 | Thymus | 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 | 1/3* |
| Parathyroid gland | 0/3 | Stomach | 0/3 |
| Hypophysis | 0/3 | Small intestine | 0/3 |
| Testis | 0/3 | Colon | 0/3 |
| Thyroid | 0/3 | Liver | 0/3 |
| Breast | 2/2# | Salivary gland* | 1/3* |
| Spleen | 0/3 | Kidney | 0/3 |
| Tonsil | 3/3* | Prostate | 10/11## |
| Endometrium | 0/2 | Cervix | 1/3* |
| Skeletal muscle | 0/3 | Skin | 3/3* |
| Nerve (sparse) | 0/3 | Mesothelium and lung | 0/3 |
| Bladder | 2/3** | N/A | N/A |

myoepithelial cells * squamous cells ** urothelial cells ## basal cells

Sensitivity

Table 4. Sensitivity of anti-p40 (BC28) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

| Pathology | # positive / total cases |
|-------------------------------|--------------------------|
| Glioblastoma | 0/1 |
| Atypical meningioma | 0/1 |
| Malignant ependymoma | 0/1 |
| Malignant oligodendroglioma | 0/1 |
| Ovarian serous adenocarcinoma | 1/1 |
| Ovarian adenocarcinoma | 1/1 |
| Islet cell carcinoma | 0/1 |
| Pancreatic adenocarcinoma | 0/1 |

| Pathology | # positive / total cases |
|--|--------------------------|
| Seminoma | 0/2 |
| Medullary carcinoma (thyroid) | 0/1 |
| Papillary carcinoma (thyroid) | 0/1 |
| Breast intraductal carcinoma | 0/1 |
| Breast invasive ductal carcinoma | 0/2 |
| Diffuse B-cell lymphoma | 0/1 |
| Lung small cell undifferentiated carcinoma | 0/1 |
| Lung squamous cell carcinoma | 74/92 |
| Lung adenocarcinoma | 13/146 |
| Lung large cell carcinoma | 1/4 |
| Lung small cell carcinoma | 0/6 |
| Lung atypical carcinoid | 0/5 |
| Bronchioloalveolar carcinoma | 0/3 |
| Adenosquamous carcinoma (lung) | 2/2* |
| Neuroendocrine carcinoma (esophagus) | 0/1 |
| Esophageal adenocarcinoma | 0/1 |
| Signet-ring cell carcinoma | 0/1 |
| Intestinal adenocarcinoma | 0/1 |
| Stromal sarcoma | 0/1 |
| Interstitialoma | 0/2 |
| Colorectal adenocarcinoma | 0/2 |
| Hepatocellular carcinoma | 0/1 |
| Hepatoblastoma | 0/1 |
| Renal clear cell carcinoma | 0/1 |
| Prostatic adenocarcinoma | 0/67 |
| Prostatic hyperplasia | 1/1 |
| Leiomyoma | 0/1 |
| Endometrial adenocarcinoma | 0/1 |
| Endometrial clear cell carcinoma | 0/1 |
| Uterine squamous cell carcinoma | 2/2 |
| Embryonal rhabdomyosarcoma | 0/1 |
| Anal malignant melanoma | 0/1 |
| Basal cell carcinoma | 1/1 |
| Squamous cell carcinoma | 1/1 |
| Neurofibroma | 0/1 |
| Retroperitoneal neuroblastoma | 0/1 |
| Epithelial malignant mesothelioma | 0/1 |

| Pathology | # positive / total cases |
|-------------------------------------|--------------------------|
| Diffuse B cell lymphoma | 0/2 |
| Hodgkin lymphoma | 0/1 |
| Anaplastic large cell lymphoma | 0/1 |
| Bladder transitional cell carcinoma | 1/1 |
| Low grade leiomyosarcoma | 0/1 |
| Osteosarcoma | 0/1 |
| Spindle cell rhabdomyosarcoma | 0/1 |
| Intermediate grade leiomyosarcoma | 0/1 |

* squamous component

Repeatability

Repeatability studies for anti-p40 (BC28) were completed to demonstrate:

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

All studies met their acceptance criteria.

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