

## p40 (M)

Prediluted Monoclonal Antibody

Control Number: 901-3066VP-101216

### VP Echelon™ Series

**Catalog Number:** AVI 3066 KG

**Description:** 6.0 ml, prediluted

**Dilution:** Ready-to-use

#### Intended Use:

For In Vitro Diagnostic Use

p40 (M) [BC28] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of p40 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

#### Summary and Explanation:

The mouse monoclonal antibody p40 [clone BC28] recognizes an epitope unique to the p40 protein and may have applications in cases where p63 has traditionally been used. To date, p63 [clone 4A4] has been a frequently used marker for lung squamous cell carcinoma (SCC), as well as for bladder, breast, prostate, and head and neck cancers. p63 [4A4] recognizes both the p63 and p40 proteins (1). As a result, p63 [4A4] has proven to be a sensitive marker for lung SCC; however, it suffers from specificity limitations due to reactivity in a subset of lung adenocarcinomas (ADC). In contrast, p40 is selectively expressed in lung SCC, offering an opportunity for improved specificity (2). p40 (M) [BC28] recognizes an epitope unique to p40, which may result in diminished reactivity in lung ADC and increased specificity.

Staining with a rabbit polyclonal anti-p40 antibody was equivalent to p63 [4A4] in sensitivity for lung SCC, but p40 exhibited markedly superior specificity due to staining in fewer cases of lung adenocarcinoma compared to p63 (1). This mouse monoclonal anti-p40 [BC28] demonstrated similar sensitivity and specificity, staining 97% (65/67) of cases of lung SCC and 0% (0/71) of cases of lung ADC (see Performance Characteristics). p40 has also been reported in combination with TTF-1 in a method to improve specificity for SCC vs. ADC, while preserving limited tissue specimens (3,4).

Changes in expression of p40 have been implicated in other neoplastic tissues, including bladder, prostate, and head and neck cancers (2,4,5). p40 (M) [BC28] was found to be a sensitive marker in each of these tissues (see Performance Characteristics). Studies have supported the routine use of p40 as an alternative for p63 (1,2-5). U.S. Patent 9,428,576 and patents pending.

#### Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. This detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Immunogen:** a synthetic peptide corresponding to amino acids 5-17 of human p40

**Clone:** BC28

**Isotype:** IgG1

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration

**Epitope/Antigen:** amino acids 5-17 of p40

**Cellular Localization:** Nuclear

**Positive Control:** Lung squamous cell carcinoma

#### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

#### Supplied As:

p40 (M) (AVI3066G) 1 x 6ml

V-Blocker (BR14001G) 1 x 6ml

**NOTE:** V-Blocker must be filled in a registered Ancillary Inline User Fillable Dispenser prior to use. V-Blocker must be registered as an "Option" in order to properly use it.

#### Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

#### Protocol Recommendations:

Using *ultraVIEW* Detection Kit

**Pretreatment Solution (recommended):** CC1

**Pretreatment Protocol:** Mild

**Primary Antibody:** Incubate for 32 minutes at 37°C.

V-Blocker is recommended to be applied prior to any detection system.

**ultraBlock (BR14001):** Incubate for 4 minutes (with appropriate Option # registered by user)

#### Technical Note:

1. Biocare's VP-Echelon Series of predilutes have been developed for use with Ventana® Medical Systems, BenchMark® XT Immunohistochemistry Staining System in combination with Ventana® Detection Kits and Ventana® Prep Kit Dispensers.

2. Application of V-Blocker prior to any detection system is highly recommended for background reduction.

3. A stronger pretreatment (CC1 Standard) and/or increase of antibody incubation time may provide better staining intensity when using other specified tissue (i.e. prostate and breast). It is the responsibility of the operator to determine optimum conditions.

#### Performance Characteristics:

Nuclear staining of p40 (M) [BC28] was observed in 97% (65/67) of cases of lung squamous cell carcinoma, with no staining observed in lung adenocarcinoma cases (n=71) (Table 1). Staining of p40 (M) was also observed in 85.5% (41/48) of cases of urothelial carcinoma and 78% (46/59) of cases of head and neck squamous cell carcinomas. In breast cancers, only myoepithelial cells in ductal carcinoma in situ (DCIS) stained with p40 (M). No cases of prostate cancer were found to be positive with p40 (M).

p40 (M) [BC28] nuclear staining was observed in the expected normal tissues: basal cells in prostate, myoepithelial cells in breast, urothelial cells in bladder (but not umbrella cells), stratified epithelial cells in skin, tonsil, esophagus and cervical mucosa, cytotrophoblasts in placenta, and extremely low staining in thymus and spleen (Table 2).

#### Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times listed are not applicable to other detection systems, as results may vary. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

#### Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

#### Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)

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#### Precautions Cont'd:

- Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)
- Microbial contamination of reagents may result in an increase in nonspecific staining.
- Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
- Do not use reagent after the expiration date printed on the vial.
- The SDS is available upon request and is located at <http://biocare.net>.

#### References:

- Bishop JA, *et al.* p40 is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol.* 2012 Mar; 25(3):405-15.
- Hibi K, *et al.* AIS is an oncogene amplified in squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 2000 May 9; 97(10):5462-7.
- Pelosi G, *et al.* p40 and thyroid transcription factor-1 immunoreactivity on small biopsies or cellblocks for typing non-small cell lung cancer: a novel two-hit, sparing-material approach. *J Thorac Oncol.* 2012 Feb; 7(2):281-90.
- Brown AF, *et al.* Tissue-preserving antibody cocktails to differentiate primary squamous cell carcinoma, adenocarcinoma, and small cell carcinoma of lung. *Arch Pathol Lab Med.* 2013 Sep; 137(9):1274-81.
- Sailer V, *et al.* Comparison of p40 and p63 expression in prostate tissues - which one is the superior diagnostic marker for basal cells? *Histopathology.* 2013 Jul; 63(1):50-6.
- Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
- Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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**Table 1:** Sensitivity of mouse monoclonal antibody p40 (M) [BC28] was determined by testing formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	Number of Specimens	Number of Positive Specimens	% Positive
Lung squamous cell carcinoma	67	65	97.0%
Lung adenocarcinoma	71	0	0%
Urothelial carcinoma	48	41	85.5%
Head and neck squamous cell carcinoma	59	46	78.0%
Breast cancer	65	18	27.6%
Prostate cancer	12	0	0%

#### Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

**Table 2:** Specificity of mouse monoclonal antibody p40 (M) [BC28] was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total tissues	Tissue	#positive / total tissues
Adrenal gland	0/3	Ovary	0/3
Bladder, urinary	2/3	Pancreas	0/3
Bone Marrow	0/1	Parathyroid	0/3
Eye	0/1	Pituitary gland	0/2
Breast	3/3	Placenta	1/3
Brain, cerebellum	0/3	Prostate	3/3
Brain, cerebral cortex	0/3	Skin	1/1
Fallopian tube	0/3	Spinal Cord	0/2
Esophagus	3/3	Spleen	0/2
Stomach	0/3	Skeletal Muscle	0/3
Intestine, small intestine	0/3	Testis	0/3
Intestine, colon	0/3	Thymus	0/3
Intestine, rectum	0/3	Thyroid	0/3
Heart	0/3	Inflammatory Tonsillitis*	3/3
Kidney	0/6	Ureter	3/3
Liver	0/3	Uterus cervix	3/3
Lung	0/3	Uterus endometrium	0/3

\*B and T cells are negative. Only normal squamous epithelium is positive.