p40 (M)

Concentrated and Prediluted Monoclonal Antibody
901-3066-052623

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3066 A, C	0.1, 1.0 mL	1:100	Van Gogh Yellow
Predilute	API 3066 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IPI 3066 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 3066 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 3066 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 3066 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3066 KG, KH	6.0, 25 mL	Ready-to-use	N/A
Q Series – For Leica BOND-III	ALI 3066 G7	7.0 mL	Ready-to-use	N/A

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. p40 is selectively expressed in lung SCC, offering an opportunity for improved specificity (1), resulting in diminished reactivity in lung ADC and increased specificity.
The mouse monoclonal anti-p40 [BC28] demonstrated high 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 (2,3).
Changes in expression of p40 have been implicated in other neoplastic tissues, including bladder, prostate, and head and neck cancers (1,2,3). 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 (1-4).
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, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are 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
Protein Concentration: Call for lot specific Ig concentration
Epitope/Antigen: amino acids 5-17 of p40
Cellular Localization: Nuclear
Positive Tissue Control: Lung squamous cell carcinoma
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative
For AVI3066KG:
p40 (M) (AVI3066G) 1 x 6mL
V-Blocker (BRI4001G) 1 x 6mL
For AVI3066KH:
p40 (M) (AVI3066H) 1 x 25mL
V-Blocker (BRI4001H) 1 x 25mL
Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTM3066 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Secondary: Incubate for 10 minutes with Val Mouse Secondary.
Linker: Incubate for 10 minutes with Val Universal Linker.
Polymer: Incubate for 10 minutes with Val Universal Polymer.
Chromogen: Incubate for 5 minutes with Val DAB.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

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Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

intelliPATH FLX Automated Slide Stainer:

IPI3066 is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI3066 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: p40
Protocol Template (Description): IHC Extras Template
Dewaxing (DS Option): DS Buffer
Antigen Retrieval (AR Option): AR1, High pH; 103°C
Reagent Name, Time, Temp.: p40, 30 min., 25°C

- Use of **Mouse Amp HRP Detection (ORI6050) is required** for the above antibody protocol. Mouse HRP Detection (ORI6007) is not recommended.

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI3066 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: p40
Protocol Template (Description): IHC Extras Template 1
Dewaxing (DS Buffer Option): DS Buffer
Antigen Retrieval (AR Option): AR1, high pH; 103°C
Block Option: Buffer
Reagent Name, Time, Temp.: p40, 59 min., 25°C

- Use of **Mouse Amp HRP Detection (OPRI6050) is required** for the above antibody protocol. Mouse HRP Detection (OPRI6007) is not recommended.

Protocol Recommendations (Ventana BenchMark XT / ULTRA):

AVI3066 is intended for use with the BenchMark XT / ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using ***ultra*View on XT / ULTRA:**

Template/Detection: ultraView DAB
Pretreatment Protocol: CC1 Mild
Primary Antibody: 32 minutes, 37°C
ultraBlock (V-Blocker BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user)
V-Blocker is recommended to be applied prior to any detection system.

- Using **OptiView on ULTRA:**

Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 64 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 32 minutes, 36°C

Protocol Recommendations (Q Series – For Leica BOND-III):

ALI3066 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Protocol Name: IHC Protocol F
Detection: Bond Polymer Refine
HIER: 30 min with ER1
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 min
Hematoxylin: 5 min

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). 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, occasional cytotrophoblasts in placenta. (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 and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

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_3) 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) (5)
2. 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 in contact with sensitive areas, wash with copious amounts of water. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

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Precautions Cont'd:
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

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.

References:
1. 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.
2. 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.
3. 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.
4. 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.
5. 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."
6. 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.

Table 1: Sensitivity and specificity of mouse monoclonal antibody p40 (M) [BC28] were 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%

Table 2: Tissue cross-reactivity 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	3/3
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.

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