p40 (M)  Concentrated and Prediluted Monoclonal Antibody
901-3066-042618

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 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).


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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated 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 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 (BR14001G) 1 x 6ml

Storage and Stability: Store at 2ºC to 8ºC. Do not use after expiration date. Any diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.


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.

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

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Description</th>
<th>Dilution</th>
<th>Diluent</th>
<th>Ready-to-use</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI 3066 A, C</td>
<td>0.1, 1.0 ml, concentrated</td>
<td>1:100</td>
<td>Van Gogh Yellow</td>
<td>N/A</td>
</tr>
<tr>
<td>API 3066 AA, H</td>
<td>6.0, 25 ml, prediluted</td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>

| Protocol Recommendations (intelliPATH and manual use) Cont’d: | | | |
|---|---|---|
| Counterstain: | | |
| - | Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha’s Bluing Solution for 1 minute. Rinse with deionized water. |

intelliPATH™ Automated Slide Stainer:

IPI3066 is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the User Manual for specific instructions for use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPBS000) may be performed following heat retrieval.

Protocol Recommendations (ONCORE Automated Slide Staining System): OAI3066 is intended for use with the ONCORE Automated Slide Staining System. Refer to the User Manual for specific instructions for use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

- Protocol Name: p40
- Protocol Template (Description): IHC Extras Template
- Pretreatment (Optional): dewaxing
- Counterstain: Hematoxylin
- Primary Antibody: 32 minutes, 37°C
- Primary Antibody: 32 minutes, 37°C
- - Use of Mouse Amp HRP Detection (ORI6050) is required for the above antibody protocol.
- - Mouse HRP Detection (ORI6007) is not recommended.

Protocol Recommendations (Ventana BenchMark XT / ULTRA Slide Staining Systems):

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

- Using ultraView on XT / ULTRA:
- Template/Detection: ultraView DAB
- Pretreatment Protocol: CC1 64 minutes
- Primary Antibody: 32 minutes, 37°C
- ultraBlock (V-Blocker BR14001): Incubate for 4 minutes (with appropriate Option if registered by user)
- V-Blocker is recommended to be applied prior to any detection system
- - Using OptiView on ULTRA:
- Template/Detection: OptiView DAB
- Pretreatment Protocol: CC1 64 minutes
- Peroxidase: Pre Primary Peroxidase Inhibitor
- Primary Antibody: 32 minutes, 36°C

Technical Note:
This antibody, for intelliPATH and manual use, has been standardized with Biocare’s MACH 4 detection system. Use TBS for washing steps.

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 and myoepithelial cells in breast, urothelial cells in bladder (but not umbrella cells), stratified epithelial cells in skin, tonsil, esophagus and cervical mucosa, occasional cytrophoblasts in placenta.

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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation.
Limitations Cont’d:
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 Controls:
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
(Na3N) 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.

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

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,

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(3):50-6.

Atlanta, GA. April 30, 1976 “Decontamination of Laboratory Sink Drains to
Remove Azide Salts.”

Workers from Occupationally Acquired Infections; Approved Guideline-Fourth

VP Echelon Series antibodies are developed solely by Biocare Medical LLC and do
not imply approval or endorsement of Biocare’s antibodies by Ventana Medical
Systems, Inc. Biocare and Ventana are not affiliated, associated or related in any
way. Ventana®, BenchMark®, ultraView and OptiView are trademarks of Roche.

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

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Number of Specimens</th>
<th>Number of Positive Specimens</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung squamous cell carcinoma</td>
<td>67</td>
<td>65</td>
<td>97.0%</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>71</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Urothelial carcinoma</td>
<td>48</td>
<td>41</td>
<td>85.5%</td>
</tr>
<tr>
<td>Head and neck squamous cell carcinoma</td>
<td>59</td>
<td>46</td>
<td>78.0%</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>65</td>
<td>18</td>
<td>27.6%</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>12</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th># positive/ total tissues</th>
<th>Tissue</th>
<th># positive/ total tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal gland</td>
<td>0/3</td>
<td>Ovary</td>
<td>0/3</td>
</tr>
<tr>
<td>Bladder, urinary</td>
<td>2/3</td>
<td>Pancreas</td>
<td>0/3</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0/1</td>
<td>Parathyroid</td>
<td>0/3</td>
</tr>
<tr>
<td>Eye</td>
<td>0/1</td>
<td>Pituitary gland</td>
<td>0/2</td>
</tr>
<tr>
<td>Breast</td>
<td>3/3</td>
<td>Placenta</td>
<td>1/3</td>
</tr>
<tr>
<td>Brain, cerebellum</td>
<td>0/3</td>
<td>Prostate</td>
<td>3/3</td>
</tr>
<tr>
<td>Brain, cerebral cortex</td>
<td>0/3</td>
<td>Skin</td>
<td>1/1</td>
</tr>
<tr>
<td>Fallopian tube</td>
<td>0/3</td>
<td>Spinal cord</td>
<td>0/2</td>
</tr>
<tr>
<td>Esophagus</td>
<td>3/3</td>
<td>Spleen</td>
<td>0/2</td>
</tr>
<tr>
<td>Stomach</td>
<td>0/3</td>
<td>Skeletal muscle</td>
<td>0/3</td>
</tr>
<tr>
<td>Intestine, small intestine</td>
<td>0/3</td>
<td>Testis</td>
<td>0/3</td>
</tr>
<tr>
<td>Intestine, colon</td>
<td>0/3</td>
<td>Thymus</td>
<td>3/3</td>
</tr>
<tr>
<td>Intestine, rectum</td>
<td>0/3</td>
<td>Thyroid</td>
<td>0/3</td>
</tr>
<tr>
<td>Heart</td>
<td>0/3</td>
<td>Inflammatory tonsillitis*</td>
<td>3/3</td>
</tr>
<tr>
<td>Kidney</td>
<td>0/6</td>
<td>Ureter</td>
<td>3/3</td>
</tr>
<tr>
<td>Liver</td>
<td>0/3</td>
<td>Uterus cervix</td>
<td>3/3</td>
</tr>
<tr>
<td>Lung</td>
<td>0/3</td>
<td>Uterus (endometrium)</td>
<td>0/3</td>
</tr>
</tbody>
</table>

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