

# BAP1

Concentrated and Prediluted Polyclonal Antibody  
901-3247-053123

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Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3247 A, B	0.1, 0.5 mL	1:50	Da Vinci Green
Predilute	API 3247 AA	6.0 mL	Ready-to-use	N/A
Q Series-- For Leica BOND-III	ALI 3247 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

BAP1 is a rabbit polyclonal antibody that is intended for laboratory use in the qualitative identification of BRCA1-associated protein 1 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:

BAP1 (BRCA-1 Associated Protein-1) is an 80 kDa protein known to function as a deubiquitinase (DUB); removing ubiquitin modifiers, which alter the function of other proteins via targeted degradation, subcellular localization, or activity (1). By removing ubiquitin, BAP1 helps regulate the function of target proteins involved in diverse cellular processes. Germline mutations in the BAP1 gene have been associated with BAP1 tumor predisposition syndrome, a condition linked to early onset and/or increased risk of multiple types of cancers including uveal melanoma, malignant mesothelioma, cutaneous melanoma, and renal cell carcinoma (2, 3).

## 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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Rabbit polyclonal

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

**Clone:** N/A

**Isotype:** IgG

**Protein Concentration:** Lot specific Ig concentration is not available.

**Epitope/Antigen:** BRCA1-associated protein 1

**Cellular Localization:** Nuclear

**Positive Tissue Control:** Mesothelioma, melanoma, normal testis

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

## Storage and Stability:

Store at 2°C to 8°C. 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 2°C to 8°C.

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

## Protocol Recommendations (intelliPATH FLX and manual use)

### Cont'd:

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** N/A

**Polymer:** Incubate for 30 minutes at RT with a secondary-conjugated polymer.

**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.

### 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 (Q Series – For Leica BOND-III):

ALI3247 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 ER2

**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:

Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

### 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<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

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## Precautions Cont'd:

Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (4).

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 into contact with sensitive areas, wash with copious amounts of water. (5)

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. Citterio E. Fine-tuning the ubiquitin code at DNA double-strand breaks: deubiquitinating enzymes at work. *Front Genet.* 2015; 6:282. Published 2015 Sep 8.

2. Murali R, Wiesner T, Scolyer RA. Tumours associated with BAP1 mutations. *Pathology.* 2013 Feb; 45(2):116-26.

3. Walpole S, *et al.* Comprehensive Study of the Clinical Phenotype of Germline BAP1 Variant-Carrying Families Worldwide. *J Natl Cancer Inst.* 2018; 110(12):1328-1341.

4. 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."

5. 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 and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Mesothelioma	8	23
Melanoma	33	40
Breast Cancer	3	27
Colon Cancer	20	43
Lung Cancer	34	48
Prostate Cancer	9	41
Adrenocortical Carcinoma	0	1
Bladder Cancer	2	2
Osteosarcoma	1	1
Meningioma	0	1
Squamous Cell Carcinoma (Esophagus)	2	3
Adenocarcinoma (Stomach)	2	2
Kidney Cancer	0	2
Liver Cancer	2	4
Lymphoma	1	3
Squamous Cell Carcinoma (Head and Neck, Oral Cavity, Tongue)	1	1
Nasopharyngeal Carcinoma	2	2
Ovary Cancer	2	2
Adenocarcinoma (Pancreas)	0	1
Adenoid Cystic Carcinoma (Head and Neck, Salivary Gland)	1	1
Squamous Cell Carcinoma (Skin)	1	1
Seminoma	1	1
Thyroid Cancer	0	2
Cervical Cancer	1	2
Endometrium Cancer	1	2

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**Table 2:** Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	3	3
Cerebellum	2	3
Adrenal	2	3
Ovary	2	3
Pancreas	4	4
Parathyroid	3	3
Pituitary	2	2
Testis	3	4
Thyroid	3	3
Breast	2	2
Spleen	3	3
Tonsil	3	4
Thymus	0	2
Bone Marrow	2	2
Lung	1	3
Heart	3	3
Esophagus	3	4
Stomach	4	4
Small Intestine	4	4
Colon	13	13
Liver	3	4
Salivary Gland	3	3
Kidney	4	4
Prostate	3	11
Uterus	4	4
Cervix	4	4
Skeletal Muscle	3	3
Skin	9	11
Peripheral Nerve	2	2
Lining Cells	2	2
Bladder	1	1
Head, Neck and Salivary Gland	0	1
Lymph Node	0	1