

H3K27me3 [C36B11]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3249-060223

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Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3249 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	API 3249 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3249 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3249 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3249 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

For In Vitro Diagnostic Use

H3K27me3 [C36B11] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of H3K27me3 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:

H3K27me3 (histone 3 lysine 27 trimethylation) is an epigenetic mark that plays a critical role in regulation of gene expression. The dysregulation of H3K27me3 is implicated in the genesis and progression of cancer. Studies indicate that H3K27me3 plays a role in the creation and maintenance of cell type-specific programs of transcriptional control for a wide variety of species and cell fates (1).

H3K27me3 expression was shown to be a significant prognostic indicator in breast, ovarian and pancreatic cancers. Low expression of H3K27me3 correlated with significantly shorter overall survival time compared to individuals with high H3K27me3 expression (2).

H3K27me3 may be a useful marker in diagnosing malignant peripheral nerve sheath tumors (MPNSTs) and providing molecular insight in progression of prostate cancer (3,4).

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. 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 monoclonal

Species Reactivity: Human; not tested

Clone: C36B11

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: H3K27me3

Cellular Localization: Nuclear

Positive Tissue Control: Ovarian cancer

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 Solution (recommended): Diva Decloaker

Pretreatment Protocol: Heat Retrieval Method: Preheat the retrieval solution to 95°C for 30 minutes and then place slides in the preheated

Protocol Recommendations (intelliPATH FLX and manual use)

Cont'd:

solution if using Decloaking Chamber Pro or Decloaking Chamber Plus. If using Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

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

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 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 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 (ONCORE™ Pro Automated Slide Staining System):

OPAI3249 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: H3K27me3 Rb

Protocol Template (Description): Rb HRP Template 1

Dewaxing (DS Buffer Option): DS2-50

Antigen Retrieval (AR Option): AR1, high pH; 101°C

Block Option: Buffer

Reagent Name, Time, Temp.: H3K27me3 Rb, 30 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):

AVI3249 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

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):

ALI3249 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: 40 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

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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 (NaN₃) 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 into 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. Arthur RK, *et al.* Evolution of H3K27me3-marked chromatin is linked to gene expression evolution and to patterns of gene duplication and diversification. *Genome Res.* 2014 July; 24:1115-24.
2. Wei Y, *et al.* Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. *Mol Carcinog.* 2008 Sep; 47(9):701-06.
3. Prieto-Granada CN, *et al.* Loss of H3K27me3 Expression Is a Highly Sensitive Marker for Sporadic and Radiation-induced MPNST. *Am J Surg Pathol.* 2016 Apr; 40(4):479-89.
4. Ngollo M, *et al.* Global Analysis of H3K27me3 as an Epigenetic Marker in Prostate Cancer Progression. *BMC Cancer.* 2017; 17:261.
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.

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Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Astrocytoma	30	33
Glioblastoma	7	7
Ovary Cancer	42	50
Breast Cancer	21	27
Colon Cancer	40	40
Lung Cancer	43	52
Prostate Cancer	35	38
Adrenocortical Carcinoma	1	1
Bladder Cancer	2	2
Meningioma	2	2
Squamous Cell Carcinoma (esophagus)	1	2
Adenocarcinoma (stomach)	2	3
Adenocarcinoma (small intestine)	0	1
Adenocarcinoma (colon & rectum)	5	6
Kidney Cancer	1	2
Liver Cancer	33	44
Lymphoma	0	3
Adenocarcinoma (head & neck, oral cavity, hard palate)	0	1
Squamous Cell Carcinoma (head & neck, oral cavity, tongue)	1	1
Nasopharyngeal Carcinoma	1	1
Adenocarcinoma (pancreas)	0	1
Adenocarcinoma (prostate)	1	2
Squamous Cell Carcinoma (skin)	1	1
Seminoma	0	2
Thyroid Cancer	2	2
Cervical Cancer	0	2
Endometrium Cancer	2	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	10	11
Cerebellum	3	3
Adrenal	2	3
Ovary	11	11
Pancreas	3	4
Parathyroid	3	3
Pituitary	2	2
Testis	3	4
Thyroid	4	4
Breast	4	4
Spleen	3	3
Tonsil	3	3
Thymus	0	3
Bone Marrow	2	2
Lung	2	3
Heart	3	3
Esophagus	3	4
Stomach	4	4
Small Intestine	4	4
Colon	12	12
Liver	8	13
Salivary Gland	3	3
Kidney	4	4
Prostate	10	12
Uterus	4	4
Cervix	2	2
Skeletal Muscle	3	3
Skin	3	3
Peripheral Nerve	2	2
Lining Cells	2	2
Bladder	1	1
Head, Neck and Salivary Gland	0	1
Lymph Node	0	1