

# Chromogranin A

Concentrated and Prediluted Cocktail Antibody  
901-010-101520

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 010 A, B, C	0.1, 0.5, 1.0 mL	1:100	Da Vinci Green
Predilute	PM 010 AA	6.0 mL	Ready-to-use	N/A
intelliPATH FLX	IP 010 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 010 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 010 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 010 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 010 G	6.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

Chromogranin A [LK2H10 + PHE5] is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of chromogranin A 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:

This antibody recognizes a protein of 68-75 kDa, identified as Chromogranin A (a protein of 439-amino acid which is encoded on chromosome 14). Although the epitopes for [LK2H10] and [PHE5] are not precisely mapped, experimental data suggests that they are different. A cocktail of LK2H10 and PHE5 is specifically designed for sensitive detection of Chromogranin A in formalin-fixed, paraffin-embedded tissues. Chromogranin A is present in neuroendocrine cells throughout the body, including the neuroendocrine cells of the large and small intestine, adrenal medulla and pancreatic islets. It is an excellent marker for carcinoid tumors, pheochromocytomas, paragangliomas and other neuroendocrine tumors. Coexpression of Chromogranin A and neuron specific enolase (NSE) is common in neuroendocrine neoplasms.

## 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

**Clone:** LK2H10 + PHE5

**Isotype:** IgG1 + IgG1

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** Chromogranin A

**Cellular Localization:** Finely granular cytoplasm

**Positive Control:** Pancreas or adrenal gland

**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 (VALENT® Automated Slide Staining Platform):

VLTM010 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-Lo 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 Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Reveal Decloaker. Refer to the Reveal 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.

**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™ Automated Slide Staining System):

OAI010 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:



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## **Protocol Recommendations (ONCORE Automated Slide Staining System) Cont'd:**

**Protocol Name:** Chromogranin

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Option):** DS Buffer

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Reagent Name, Time, Temp.:** Chromogranin, 30 min., 25°C

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

OPA1010 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:** Chromogranin

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Buffer Option):** DS Buffer

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** Chromogranin, 30 min., 25°C

## **Protocol Recommendations (Ventana BenchMark ULTRA):**

AVI010 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 16 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 16 minutes, 36°C

## **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](http://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) (7)

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. (8)

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.

## **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. Barthez PY, *et al.* Pheochromocytoma in dogs: 61 cases (1984-1995). J Vet Intern Med. 1997 Sept-Oct; 11(5):272-8.
2. Terada T, *et al.* Endocrine cells in intraductal papillary-mucinous neoplasms of the pancreas. A histochemical and immunohistochemical study. Virchows Arch. 1997 Jul;431(1):31-6.
3. Declich P, *et al.* What type of chromogranin does PHE5 monoclonal antibody react with? Virchows Arch. 1997 Jun;430(6):509-10.
4. Burke AP, *et al.* Carcinoids of the jejunum and ileum: an immunohistochemical and clinicopathologic study of 167 cases. Cancer. 1997 Mar 15;79(6):1086-93.
5. Angelsen A, *et al.* Neuroendocrine differentiation in carcinomas of the prostate: do neuroendocrine serum markers reflect immunohistochemical findings? Prostate. 1997 Jan 1;30(1):1-6.
6. Meng Y, Li W, Yu J. A pathological study on phenotype differentiation and its significance in pulmonary large cell carcinoma. Zhonghua Bing Li Xue Za Zhi. 1996 Dec;25(6):347-50.
7. 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."
8. 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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