

Anti-Chromogranin A (LK2H10) Primary Antibody

REF 760-2519

05267056001

IVD  50

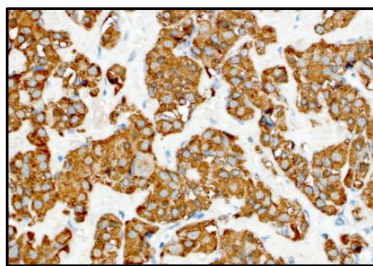


Figure 1. Anti-Chromogranin A (LK2H10) antibody staining medullary thyroid carcinoma.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

Anti-Chromogranin A (LK2H10) Primary Antibody (anti-Chromogranin A (LK2H10) antibody) is a mouse monoclonal antibody produced against the major member of the granin family of acidic secretory glycoproteins, chromogranin A, that is expressed in all endocrine and neuroendocrine cells.¹ The chromogranin A protein is expressed by the following tissues: adrenal (medulla), paraganglia, pancreas (islets), thyroid (c-cells), parathyroid, pituitary (anterior), endocrine cells of the stomach, small bowel, colon and lungs.^{2,3} The chromogranin A protein is expressed in tumors of neuroendocrine origin including: pheochromocytomas, pituitary adenomas, islet cell tumors, medullary thyroid carcinomas, carcinoids and Merkel cell tumors.³

PRINCIPLE OF THE PROCEDURE

Anti-Chromogranin A (LK2H10) antibody may be used as the primary antibody for immunohistochemical staining of paraffin tissue sections and optimally diluted for use with VENTANA detection kits and automated slide stainers. For more detailed information on instrument operation, refer to the appropriate VENTANA Automated Slide Stainer Operator's Manual.

The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and evaluation of proper negative and positive controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

REAGENT PROVIDED

Anti-Chromogranin A (LK2H10) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-Chromogranin A (LK2H10) antibody contains approximately 5 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.1 M phosphate buffered saline with a carrier protein and 0.05% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 5 mg/mL. Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-Chromogranin A (LK2H10) antibody is a IgG₁ purified mouse monoclonal.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: Principles of the Procedure, Materials and Reagents Needed but Not Provided, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Interpretation of Results, General Limitations and Troubleshooting.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

STORAGE

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.⁴ Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

Ventana recommends the use of Superfrost Plus slides, or equivalent.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-Chromogranin A (LK2H10) antibody with *ultraView* Universal DAB detection kit.

| Procedure Type | Method | | |
|---------------------------------------|---------------------------|------------------|-----------------------|
| | GX | XT | ULTRA |
| Deparaffinization | Selected | | |
| Cell Conditioning (Antigen Unmasking) | CC1, Mild | CC1, Mild | CC1, 36 minutes, 95°C |
| Antibody (Primary) | 12 minutes, 37°C | 12 minutes, 37°C | 12 minutes, 36°C |
| Counterstain | Hematoxylin II, 4 minutes | | |
| Post Counterstain | Bluing, 4 minutes | | |

Table 2. Recommended Staining Protocol for anti-Chromogranin A (LK2H10) antibody with OptiView DAB IHC detection kit.

| Procedure Type | Method | | |
|---------------------------------------|---------------------------|-------------------|------------------------|
| | GX | XT | ULTRA |
| Deparaffinization | Selected | | |
| Cell Conditioning (Antigen Unmasking) | CC1 24 minutes | CC1 24 minutes | CC1, 24 minutes, 100°C |
| Antibody (Primary) | 4 minutes, 37°C | 4 minutes, 37°C | 4 minutes, 36°C |
| Counterstain | Hematoxylin II, 4 minutes | | |
| Post Counterstain | Bluing, 4 minutes | | |

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁵

QUALITY CONTROL PROCEDURES

Negative Reagent Control

Ventana Medical Systems, Inc. strongly recommends a negative reagent control be used to stain an adjacent section of the patient specimen tissue on a separate slide from the anti-Chromogranin A (LK2H10) antibody stained slide. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001) is recommended for use in place of the primary antibody to evaluate non-specific staining. The staining protocol for the negative reagent control antibody should be the same as that for the primary antibody.

Positive Control Tissue

Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. This practice helps to identify a failure to apply primary antibody or other critical reagent to the patient test slide. A tissue with weak positive staining is more suitable for optimal quality control. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining elements and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy, or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid to determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimen should be considered invalid.

An example of a positive control tissue is normal appendix that is positive for anti-Chromogranin A (LK2H10) antibody. The positive tissue control should exhibit strong

cytoplasmic staining in neuroendocrine cells and weak to moderate staining intensity of the normal ganglion cells and axons of the nerve plexus of the appendix.

Negative Control Tissue

A negative tissue control would be a pre-qualified case of normal appendix in which the appendiceal columnar epithelial cells are negative to the anti-Chromogranin A (LK2H10) antibody. The negative tissue control should be used only to monitor performance of processed tissues, test reagents and instruments and not as an aid in formulating a specific diagnosis of patient samples.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for anti-Chromogranin A (LK2H10) antibody is cytoplasmic.

Interpretation of Results

The VENTANA automated immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody. Refer to the appropriate detection kit package insert for expected color reactions. It is recommended that a qualified pathologist experienced in immunohistochemistry procedures evaluate positive and negative controls before interpreting results.

Positive Tissue Control

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Refer to the package insert of the detection kit used for expected color reactions. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

If the positive tissue control fails to demonstrate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

SPECIFIC LIMITATIONS

The antibody has been optimized for a 12 minutes (*ultraView* Universal DAB Detection Kit) or 4 minutes (OptiView DAB IHC Detection Kit) incubation time in combination with BenchMark automated slide stainers. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁵

1. The antibody, in combination with VENTANA detection kits and accessories, detects antigen that survive routine formalin fixation, tissue processing and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.
2. This antibody demonstrates weaker staining in pheochromocytomas when antigen retrieval is selected.
3. Antigen retrieval is required for optimal staining of medullary thyroid carcinomas.
4. OptiView DAB IHC Detection Kit is generally more sensitive than *ultraView* Universal DAB Detection Kit. The user must validate the results obtained with this reagent and detection systems.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 3 and Table 4 and in the Repeatability section.

Table 3. Analytical Sensitivity/Specificity of anti-Chromogranin A (LK2H10) antibody staining in FFPE normal tissues.

| Tissue | # positive / total cases | Tissue | # positive / total cases |
|--------------------------------|--------------------------|------------------------------|--------------------------|
| Cerebrum ^a | 2/3 | Thymus | 0/3 |
| Cerebellum ^b | 3/3 | Myeloid (bone marrow) | 0/3 |
| Adrenal gland ^c | 2/3 | Lung ^h | 1/3 |
| Ovary | 0/3 | Heart | 0/3 |
| Pancreas ^d | 4/4 | Esophagus | 0/3 |
| Parathyroid gland ^e | 3/3 | Stomach ^h | 3/3 |
| Hypophysis ^f | 4/4 | Small intestine ^h | 3/3 |
| Testis ^g | 2/3 | Colon ^h | 11/11 |
| Thyroid ⁱ | 1/17 | Liver | 0/3 |
| Breast | 0/3 | Salivary gland | 0/3 |
| Spleen | 0/3 | Kidney | 0/3 |
| Tonsil | 0/3 | Prostate ^h | 3/3 |
| Endometrium | 0/3 | Cervix ^h | 1/3 |
| Skeletal muscle | 0/3 | Skin | 0/3 |
| Nerve (sparse) | 0/3 | Mesothelium and lung | 0/3 |

^a Neurons; ^b Granular Layer Cell Neurons (rare cells⁶); ^c Chromaffin Cells; ^d Islets of Langerhans; ^e Chief Cells; ^f Anterior Pituitary Epithelial Cells; ^g Spermatogenic Cells; ^h Neuroendocrine Cells; ⁱ C-cells, focal staining

Table 4. Analytical Sensitivity/Specificity of anti-Chromogranin A (LK2H10) antibody staining in a variety of FFPE neoplastic tissues.

| Pathology | # positive / total cases |
|---|--------------------------|
| Glioblastoma | 0/1 |
| Meningioma | 0/1 |
| Anaplastic ependymoma | 0/1 |
| Oligodendroglioma* | 1/1 |
| Pituitary adenoma | 1/2 |
| Parathyroid adenoma | 2/2 |
| Adrenal cortical adenocarcinoma | 1/12 |
| Pheochromocytoma | 32/33 |
| Adrenocortical adenoma | 2/42 |
| Ovarian serous papillary adenocarcinoma | 1/2* |
| Pancreatic neuroendocrine neoplasm | 0/3 |
| Pancreatic adenocarcinoma | 0/1 |

| Pathology | # positive / total cases |
|------------------------------------|--------------------------|
| Seminoma | 0/1 |
| Embryonal carcinoma | 0/1 |
| Thyroid follicular adenoma | 0/4 |
| Thyroid medullary carcinoma | 28/41 |
| Thyroid papillary carcinoma | 0/3 |
| Thyroid follicular carcinoma | 0/2 |
| Thyroid clear cell carcinoma | 0/2 |
| Thyroid undifferentiated carcinoma | 0/2 |
| Breast invasive ductal carcinoma | 1/3* |
| Lung small cell carcinoma | 1/3 |
| Lung squamous cell carcinoma | 0/1 |
| Lung adenocarcinoma | 0/1 |
| Esophageal squamous cell carcinoma | 0/1 |
| Esophageal adenocarcinoma | 0/1 |
| Gastric adenocarcinoma | 1/1* |
| Colorectal adenocarcinoma | 5/41** |
| GIST | 1/3* |
| Rectal carcinoid tumor | 1/2 |
| Hepatocellular carcinoma | 0/1 |
| Hepatoblastoma | 0/1 |
| Renal clear cell carcinoma | 0/1 |
| Prostatic adenocarcinoma | 2/2* |
| Prostatic urothelial carcinoma | 0/1 |
| Leiomyoma | 0/1 |
| Endometrial adenocarcinoma | 0/1 |
| Endometrial clear cell carcinoma | 0/1 |
| Cervical squamous cell carcinoma | 0/2 |
| Embryonal rhabdomyosarcoma | 0/1 |
| Anal malignant melanoma | 0/1 |
| Basal cell carcinoma | 1/1 |
| Squamous cell carcinoma (skin) | 0/1 |
| Neurofibroma | 0/1 |
| Retroperitoneal neuroblastoma | 1/1 |
| Malignant mesothelioma | 0/1 |
| B-cell lymphoma; NOS | 0/3 |
| Hodgkin lymphoma | 0/1 |
| Bladder urothelial carcinoma | 0/1 |

| Pathology | # positive / total cases |
|------------------------------|--------------------------|
| Leiomyosarcoma | 0/2 |
| Pleomorphic rhabdomyosarcoma | 0/1 |

*Focal staining, **Focal staining in areas of neuroendocrine differentiation

Repeatability

Repeatability studies for anti-Chromogranin A (LK2H10) antibody were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark ULTRA instrument.
- Intra-platform reproducibility on the BenchMark GX instrument, BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark GX instrument, BenchMark XT instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

1. Hendy GN, et al. Chromogranin A. Clin Invest Med. 1995 Feb 18(1): 47-65
2. Wilson BS, Lloyd RV. Detection of Chromogranin in Neuroendocrine cells with a monoclonal antibody. Am J Clin Pathol 115(3): 458-468, 1984.
3. Helman L, et al. Chromogranin A Expression in Normal and Malignant Human Tissues. J Clin Investigations. 1988 Aug (82): 686-690.
4. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
5. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
6. Muñoz, D.G., 1990. Monodendritic neurons: a cell type in the human cerebellar cortex identified by chromogranin A-like immunoreactivity. Brain Res. 528 (2), 335-338.

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