Anti-Chromogranin A (LK2H10) Primary Antibody

REF 760-2519

05267056001

IVD Σ 50

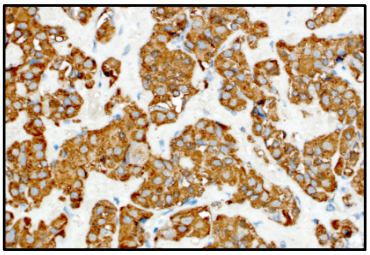

Figure 1. Anti-Chromogranin A (LK2H10) antibody staining medullary thyroid carcinoma.

INTENDED USE

Anti-Chromogranin A (LK2H10) Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of chromogranin A by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

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

Chromogranin A, a member of the granin family of proteins, is a 439 amino acid-long acidic glycoprotein with a molecular weight of 48-52 kDa.[1,2] Chromogranin A constitutes the major portion of neurosecretory granules, and is expressed by normal and tumor cells of endocrine and neuroendocrine tissues, as well as by some cancer cells that undergo neuroendocrine differentiation.[3,4] Chromogranin A is produced as a pre-protein that undergoes several post-translational modifications, including glycosylation, sulfation, phosphorylation, and proteolytic cleavage.[1,2] It is released into blood either as a full-length protein, or in multiple fragments that are involved in the regulation of the cardiovascular system, metabolism, innate immunity, angiogenesis, and tissue repair.[1,2]

Several studies demonstrate the expression of chromogranin A in tumors of neuroendocrine lineage, and its absence in non-neuroendocrine tumors.[3-10] Chromogranin A is considered the most specific marker for neuroendocrine differentiation in current clinical practice.[11,12] Thus, the detection of chromogranin A by immunohistochemistry (IHC) with anti-Chromogranin A (LK2H10) Primary Antibody (anti-Chromogranin A (LK2H10) antibody) may be used to aid in the diagnosis of neuroendocrine tumors.

Anti-Chromogranin A (LK2H10) antibody is a mouse monoclonal antibody produced against chromogranin A. The staining pattern for this antibody is cytoplasmic. It may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

Anti-Chromogranin A (LK2H10) antibody binds to Chromogranin A in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001) or OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the respective detection kit method sheet for further instructions.

MATERIAL 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 phosphate buffered saline with a carrier protein and 0.05% ProClin 300, a preservative.

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 mouse monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

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 method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

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 FFPE 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.[13] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. 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.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[14,15]

8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

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 the tables below 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 instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 760-2519.

Table 2. Recommended staining protocol for anti-Chromogranin A (LK2H10) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA 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		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for anti-Chromogranin A (LK2H10) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 24 minutes	CC1, 24 minutes	ULTRA CC1, 24 minutes, 100°C
Pre Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	4 minutes, 37°C	4 minutes, 37°C	4 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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."[16]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-Chromogranin A (LK2H10) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control tissue.

Control tissue should be fresh autopsy, biopsy, or surgical specimen, 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 performance of reagents and instruments, not as an aid to determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of 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.

STAINING INTERPRETATION / EXPECTED RESULTS

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

SPECIFIC LIMITATIONS

This antibody demonstrates weaker staining in pheochromocytomas when antigen retrieval is selected.

Antigen retrieval is required for optimal staining of medullary thyroid carcinomas.

OptiView detection system is generally more sensitive than *ultra*View detection system.

The user must validate the results obtained with this reagent and detection systems.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. 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	Heart	0/3
Cerebellum [b]	3/3	Esophagus	0/3
Adrenal gland [c]	3/5	Stomach [h]	3/3
Ovary	0/3	Small intestine [h]	3/3
Pancreas [d]	4/4	Colon [h]	11/11
Lymph node	0/3	Liver	0/3
Parathyroid gland [i]	3/3	Salivary gland	0/3
Pituitary gland [e]	4/4	Kidney	0/3
Testis [f]	2/3	Prostate [h]	3/3
Thyroid [g]	1/17	Bladder	0/3
Breast	0/3	Endometrium	0/3
Spleen	0/3	Cervix [h]	1/3
Tonsil	0/3	Skeletal muscle	0/3
Thymus	0/3	Skin	0/3
Bone marrow	0/3	Nerve	0/3
Lung [h]	1/3	Mesothelium	0/4

[a] Neurons; [b] Granular Layer Cell Neurons (rare cells[17]); [c] Chromaffin Cells; [d] Islets of Langerhans; [e] Anterior Pituitary Epithelial Cells, [f] Stromal support cells; [g] C-cells, focal staining; [h] Neuroendocrine Cells; [i] Chief Cells

Table 5. Sensitivity/Specificity of anti-Chromogranin A (LK2H10) antibody staining in a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum) [a]	1/1
Cortical adenocarcinoma (Adrenal gland)	1/12
Pheochromocytoma (Adrenal gland)	32/33
Adrenocortical adenoma (Adrenal gland)	2/42
Adenoma (Parathyroid gland)	2/2
Adenoma (Pituitary gland)	2/2
Serous carcinoma (Ovary) [a]	1/2
Neuroendocrine neoplasm (Pancreas)	0/3
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Follicular adenoma (Thyroid)	0/4
Medullary carcinoma (Thyroid)	28/41
Papillary carcinoma (Thyroid)	0/3
Follicular carcinoma (Thyroid)	0/2
Clear cell carcinoma (Thyroid)	0/2
Undifferentiated carcinoma (Thyroid)	0/2
Invasive ductal carcinoma (Breast) [a]	1/3
Small cell carcinoma (Lung)	1/3
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach) [a]	1/1
Gastrointestinal stromal tumor (GIST) (Small intestine) [a]	1/1
Adenocarcinoma (Colon)	5/40
Gastrointestinal stromal tumor (Colon) [a]	0/1
Adenocarcinoma (Rectum) [b]	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Well-differentiated neuroendocrine tumor (Carcinoid tumor, Rectum)	1/2
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1

Pathology	# positive / total cases
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate) [a]	2/2
Leiomyosarcoma (Uterus)	0/1
Carcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	0/1
Neuroendocrine carcinoma (Skin)	0/1
Neurofibroma (Nerve)	0/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	0/1
Pleomorphic rhabdomyosarcoma (Peritoneum)	0/1
Hodgkin lymphoma (Lymph node)	0/1
B-cell lymphoma, NOS	0/3
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

[a] Focal staining, [b] Focal staining in areas of neuroendocrine differentiation

Precision

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

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of anti-Chromogranin A (LK2H10) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
E	Updates to Specimen Preparation, Warnings and Precautions, Staining Procedure, Analytical Performance, and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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