Anti-Chromogranin A (LK2H10) Primary Antibody

Catalog Number 760-2519

INDICATIONS AND USE

Intended Use

This antibody is intended for *in vitro* diagnostic use.

Ventana Medical Systems' (Ventana) anti-Chromogranin A (LK2H10) Primary Antibody contains a mouse monoclonal antibody (IgG1) directed against a protein found in the secretory granules of most endocrine cells.[1] The Chromogranin A protein is widely expressed in neuronal tissues and in the secretory granules of human endocrine cells. Chromogranin A is also expressed by adrenal (medulla), parathyroid, anterior pituitary cells, islet cells of the pancreas, gastrointestinal cells, bronchial endocrine cells, and thyroid c-cells. Chromogranin A is expressed in tumors of neuroendocrine origin, including pheochromocytomas, pituitary adenomas, islet cell tumors, medullary thyroid carcinomas, carcinoids and Merkel cell tumors. This reagent may be used to aid in the identification of cells of normal or neoplastic neuroendocrine lineage. The antibody is intended for qualitative staining in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer. Anti-Chromogranin A (LK2H10) specifically binds to the chromogranin A protein located in the secretory granules of normal and neoplastic neuroendocrine cells. Unexpected antigen expression or loss of expression may occur, especially in neoplasms. Occasionally stromal elements surrounding heavily stained tissue and/or cells will show immunoreactivity.

The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Caution: U.S. Federal law restricts this device to sale by or on the order of a physician.

Summary and Explanation

Anti-Chromogranin A (LK2H10) is an antibody which binds to chromogranin A in paraffin embedded tissue sections.

Principles and Procedures

Anti-Chromogranin A (LK2H10) may be used as the primary antibody for immunohistochemical staining of paraffin tissue sections. In general, immunohistochemical staining allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and cover slipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Anti-Chromogranin A (LK2H10) is optimally diluted for use with Ventana detection kits and automated slide stainers. Each step in the staining protocol includes incubation for a precise time at a specific temperature. At the end of each incubation step, the sections are rinsed by the Ventana automated slide stainer to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps. To minimize evaporation of the aqueous reagents from the specimen containing slide a coverslip solution is applied in the slide stainer. Staining is completed after incubation with a substrate chromogen and optional counterstaining. 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.

MATERIALS AND METHODS

Reagents Provided

One dispenser of Anti-Chromogranin A (LK2H10) Primary Antibody contains 5 mL of reagent. The dispenser contains approximately 5 µg (1 µg/mL) of a mouse monoclonal antibody directed against chromogranin A present in tissue. The antibody is diluted in 0.1 M phosphate buffered saline with a carrier protein and a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL.

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on a Ventana automated slide stainer in combination with Ventana detection kits. No reconstitution, mixing, dilution, or titration is required.

Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls. (See Quality Control Procedures section.)

Materials and Reagents Needed But Not Provided

The following reagents and materials may be required for staining but are not provided with the Primary Antibody:

1. Positive and negative tissue controls
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 70° C ± 5° C
4. Bar code labels (appropriate bar code labels for negative control and the primary antibody being tested)
5. 10% neutral buffered formalin
6. Staining jars or baths
7. Timer
8. Xylene
9. Ethanol or reagent alcohol
10. Deionized or distilled water
11. ES®, NexES IHC®, BenchMark®, BenchMark® XT automated slide stainers
12. iVIEW™ DAB (preferred), AEC, V Red (ALK PHOS), Enhanced V Red detection kits
13. Detection Kit specific software (ES automated slide stainer only)
14. APK Wash Solution (ES and NexES IHC automated slide stainers)
15. Liquid Coverslip™ solution (ES and NexES IHC automated slide stainers)
16. EZ Prep™ solution (BenchMark and BenchMark XT automated slide stainers)
17. Reaction Buffer (BenchMark and BenchMark XT automated slide stainers)
18. LCS (BenchMark and BenchMark XT automated slide stainers)
19. Hematoxylin Counterstain
20. Bluing Reagent
21. Negative Control Reagent
22. Rabbit Negative Control
23. Mounting medium
24. Cover glass
25. Light microscope (20-80X)

Storage and Handling

Store at 2-8° C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert.

To ensure proper reagent delivery after every run, the cap must be replaced and the dispenser must be immediately placed 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 for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Your local Ventana office should be contacted immediately if there is an indication of reagent instability.

Specimen Collection and Preparation for Analysis

Routinely processed, formalin fixed paraffin embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer (see Materials and Reagents Needed, But Not Provided section). The recommended tissue fixative is 10% neutral buffered formalin.[2] Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut at approximately 5 µm thickness and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but not longer than 24 hours) in a 70° C ± 5° C oven.

Manual Deparaffinization Procedure

Required when using the ES or NexES IHC automated slide stainers or if deparaffinizaion is not selected on the BenchMark or the BenchMark XT automated slide stainers:

1. For instructions on when to label slides with bar code label, refer to the Instructions for Use section of the specific automated slide stainer.

2. Immerse the slides sequentially in 3 xylene baths for 5 ± 1 minutes each.
3. Transfer the slides to 100 % ethanol and immerse sequentially in 2 baths for 3 ± 1 minutes each.
4. Transfer the slides to 95% ethanol and immerse them in a bath of this solution for 3 ± 1 minutes.
5. Transfer the slides to 80% ethanol and immerse them in this solution for 3 ± 1 minutes.
6. Transfer the slides to a bath of deionized or distilled water and dip a minimum of 10 times.
7. Transfer slides to APK Wash (1X) solution or buffer solution as appropriate. For APK Wash solution, the slides should remain until you are ready to perform the staining run. For buffer solution, the slides should remain until you are ready to perform the antigen unmasking procedure. Do not allow the slides to dry.

Slides stained on the BenchMark or BenchMark XT can be deparaffinized on the instrument. If this option is selected, barcode slides and place them on the instrument. If the option is not selected follow the Manual Deparaffinization Procedure above.

WARNINGS AND PRECAUTIONS

1. Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials (example: xylene or formaldehyde).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.
6. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change.
7. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is ProClin 300. Symptoms of overexposure to ProClin 300 include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 300 in this product is 0.05% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals.

INSTRUCTIONS FOR USE

Step by Step Procedure

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. Recommended staining protocols for the automated slide stainers are listed below in Table 1. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the Operator's Manual. Other operating parameters have been preset at the factory.

Table 1. Recommended Staining Protocols for Anti-Chromogranin A (LK2H10)

Procedure Type	Platform/Method	
	ES or NexES IHC	BenchMark or BenchMark XT
Deparaffinization	Off Line	Selected
Cell Conditioning (Anitgen Unmasking)	None Required	None Required
Enzyme (Protease)	None Required	None Required
Antibody (Primary)	16 minutes recommended	16 minutes recommended
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	2 to 4 minutes	2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

The procedures for staining on the Ventana automated slide stainer are as follows. For more detailed instructions see Operator's Manual. Note: BenchMark XT has expanded protocol options. Refer to the Operator's Manual for further instructions.

ES and NexES IHC Automated Slide Stainers

Antigen Unmasking Required:

1. Slides are deparaffinized through a series of xylene and gradient alcohols to water and appropriate buffer. Perform antigen unmasking procedure and transfer slides to APK Wash (1X).
2. Load the primary antibody and appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place them on the automated slide stainer. Check bulk fluids and waste.
3. Dry the painted end of the slide and then apply slide bar code label which corresponds to the antibody protocol to be performed.
4. Load the deparaffinized, antigen unmasked labeled slides from the APK Wash (1X) Avoid tissue drying.

Antigen Unmasking Not Required:

1. Apply bar code labels. Slides are then deparaffinized through a series of xylene and gradient alcohols to water.
2. Load the primary antibody and appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place them on the automated slide stainer.
3. Load the deparaffinized labeled slides from the APK Wash (1X). Avoid tissue drying.

BenchMark or BenchMark XT Automated Slide Stainers

1. Apply slide bar code label which corresponds to the antibody protocol to be performed.
2. Load the primary antibody and appropriate detection kit dispensers and required accessory reagent onto the reagent tray and place them on the automated slide stainer. Check bulk fluids and waste.
3. Load the slides onto the automated slide stainer.

For All Instruments:

1. Start the staining run.
2. At the completion of the run, remove the slides from the instrument.
3. For *i*VIEW DAB and Ventana Red kit, wash in a mild dishwashing detergent or alcohol to remove the coverslip solution; dehydrate, clear, and coverslip with permanent mounting media in the usual manner.
4. For AEC chromogen, do not dehydrate and clear. Mount AEC with aqueous mounting medium. The stained slides should be read within two to three days of staining, and are stable for at least two years if properly stored at room temperature (15 to 25° C).

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. This tissue may contain both positive and negative staining cells or tissue components 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. Such tissues may monitor all steps of the procedure, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. An example of a positive control with Anti-Chromogranin A (LK2H10) Primary Antibody is pancreas. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly.

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

Negative Tissue Control

The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens should be considered invalid.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local Ventana office immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the patient samples.

Negative Reagent Control

A negative reagent control must be run for every specimen to aid in the interpretation of results. A negative reagent control is used in place of the primary antibody to evaluate nonspecific staining. The slide should be stained with Negative Control Reagent (mouse) or Rabbit Negative Control, as appropriate. If an alternative negative reagent control is used, dilute to the same concentration as the primary antibody antiserum with Ventana Antibody Diluent. The diluent alone may be used as an alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should equal the primary antibody incubation period.

When panels of several antibodies are used on serial sections, a negative reagent control on one slide may serve as a negative or non specific binding background control for other antibodies.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known immunohistochemistry performance characteristics representing known positive and negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[3] or the NCCLS Approved Guideline[4] or both documents). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Tissues listed in the Summary of Expected Results section are suitable for assay verification.

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. A qualified pathologist experienced in immunohistochemistry procedures must 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.

Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from excessively formalin fixed tissues. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

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. If necessary, use a panel of antibodies to aid in the identification of false negative reactions (see Summary of Expected Results section). 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.

LIMITATIONS

General Limitations

1. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to produce the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. Ventana provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.[5] Contact your local Ventana office with documented unexpected reactions.
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[6]
9. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of autoantibodies or natural antibodies.
10. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.[7]
11. As with any immunohistochemisty test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

Specific Limitations

1. The antibody has been optimized for a 16 minute incubation time in combination with Ventana detection kits and the Ventana automated slide stainer. 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” .[8]
2. The antibody, in combination with Ventana detection kits and accessories, detects antigen that survives routine formalin fixation, tissue processing and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

SUMMARY OF EXPECTED RESULTS

1. Specificity of Anti-Chromogranin A (LK2H10) was determined by a study that showed appropriate staining in a variety of formalin fixed, paraffin embedded tissues. The following results were found in normal human tissues: for breast, spinal cord, esophagus, kidney, skin, tonsil, liver and muscle, 0 out of 3 cases were positive; for intestine, 3 out of 3 were positive in the Paneth cells; for pancreas, 3 out of 3 were positive in the islet of langerhans; and for thyroid, 1 out of 3 were positive in the C cells.
2. Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity

weakens Chromogranin detection by Anti-Chromogranin A (LK2H10) and may generate false negative results.

3. Intra run reproducibility of staining was determined by five slides containing the same tissue. Five of five slides stained positively. All slides stained with same staining intensity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium, and high antigen density in a single run.
4. Inter run reproducibility of staining was determined by staining slides containing the same tissue on five different instrument runs. Five of five slides stained positively. All slides stained with similar staining intensity. Users should verify between run reproducibility results by staining several sets of serial sections with low, medium and high antigen density on different days.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label. If the slide is labeled properly, other positive controls run on the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific antibody staining is too intense, the run should be repeated with incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
6. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
7. For corrective action, refer to the Step By Step Procedure section, the Automated Slide Stainer Users Manual or contact your local Ventana office.

REFERENCES

1. Wilson BS, Lloyd RV. Detection of Chromogranin in Neuroendocrine cells with a monoclonal antibody. Am J Clin Pathol 115(3): 458-468, 1984.
2. Sheehan DC, Hrapchak BB. Theory and practice of histotechnology, 2nd Edition. The C.V. Mosby Company, St. Louis, 1980.
3. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2001.
4. NCCLS. Quality Assurance for Immunocytochemistry: Approved Guideline. NCCLS document MM4-A- (ISBN 1-56238-396-5). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
5. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 66(4): 194-199, 1991.
6. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol 73(5): 626-632, 1980.
7. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med 14: 767, 1983.
8. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.ventanamed.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany

CE