VENTANA CLDN18 (43-14A) Assay

REF 790-7027

08504148001

IVD Σ 50

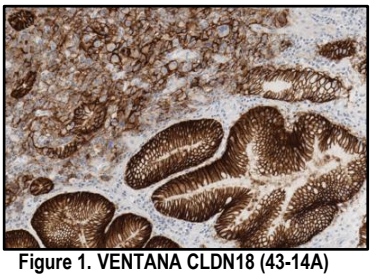

Figure 1. VENTANA CLDN18 (43-14A) Assay expression on gastric adenocarcinoma tissue.

INTENDED USE

VENTANA CLDN18 (43-14A) Assay is intended for the immunohistochemical detection of the Claudin 18 (CLDN18) protein in formalin-fixed, paraffin-embedded (FFPE) neoplastic tissues stained with BenchMark IHC/ISH instruments.

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

VENTANA CLDN18 (43-14A) Assay is a mouse monoclonal antibody (clone 43-14A) produced against Claudin 18 proteins and it detects both Claudin 18.1 and 18.2 isoforms. This assay produces membranous staining.

The transmembrane Claudin 18 proteins comprise a major component of tight junctions, which regulate paracellular barrier permeability and membrane polarity. CLDNs control the selective transport of ions across cells and maintain the integrity of epithelial and endothelial layers.[1,2] In mammals, 27 Claudin family members have been identified.[3] The predicted protein structure of CLDN includes four transmembrane segments[4] with two extracellular loops, and the N- and C- termini localized in the cytoplasm. Claudin 18 gene is a 785-base pair cDNA coding for a 261 amino acid protein of ~27.7 kDa molecular weight. Claudin 18 has two isoforms (CLDN18.1 and 18.2). CLDN18.2 differs from CLDN18.1 in the N-terminal 69 amino acids, including the first extracellular loop.[5]

Unlike most of the CLDN family members, CLDN18 does not exhibit broad tissue expression. Isoforms of CLDN18 can be detected short-lived in differentiated epithelial cells of the normal gastric mucosa and pneumocytes in normal lung tissue, in a variety of advanced and metastatic gastrointestinal cancers, including gastric, esophageal, pancreatic, biliary duct cancers, and in non-small-cell lung cancer.[6-12]

PRINCIPLE OF THE PROCEDURE

VENTANA CLDN18 (43-14A) Assay is a mouse monoclonal primary antibody which binds to CLDN18 protein in paraffin-embedded tissue sections. The specific antibody can be visualized by using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the OptiView DAB IHC Detection Kit package insert for further information.

MATERIAL PROVIDED

VENTANA CLDN18 (43-14A) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA CLDN18 (43-14A) Assay contains approximately 15 µg of mouse monoclonal antibody.

The antibody is diluted in 0.05 M Tris buffered saline, EDTA, Brij-35 and 0.05% sodium azide, a preservative. There is a trace amount of bovine serum albumin, carrier protein.

Specific antibody concentration is approximately 3 µg/mL.

VENTANA CLDN18 (43-14A) Assay is a mouse monoclonal antibody IgG2b produced as cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert 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 General 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 package insert 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. Drying oven capable of maintaining a temperature of 60°C ± 5°C
4. Bar code labels
5. Xylene (Histological grade)
6. Ethanol or reagent alcohol (Histological grade)
   - 100% solution: Undiluted ethanol or reagent alcohol
   - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
   - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
7. Deionized or distilled water
8. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
9. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
10. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
11. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
12. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
13. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
14. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
15. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
16. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
17. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
18. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
19. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
20. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
21. Light microscope
22. Absorbent wipes

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] with 6 to 48 hour fixation time.

Tissue sections should be cut at approximately 4 µm thick and mounted on positively charged glass slides. Tissue sections can be cut at 3 to 6 µm for this assay.

Before staining, the cut slides should be dried completely either at room temperature (air-dried) or offline baking (baked in oven) at 60°C for 60 minutes

Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised due to environmental factors during extended storage.[17-20] Recommendations for the extended storage of unstained slides can be found in the Specific Limitations section.

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. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
3. For professional use only.
4. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining of any IHC assay (for example, lack of primary antibody or counterstain on the tissue). Ask your Roche representative for a copy of "Impacts of Environmental Stresses on IHC Positively Charged Slides" to better understand how to use these types of slides.
5. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. 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.
9. Consult local and/or state authorities with regard to recommended method of disposal.
10. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
11. 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.

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 for the 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 VENTANA CLDN18 (43-14A) Assay with OptiView DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Baking	Not Selected	Not Selected	Not Selected
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes	CC1, 64 minutes	ULTRA CC1 64 minutes, 100°C
Pre-antibody Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary) or Negative Control (Monoclonal)	16 minutes, 37°C	32 minutes, 37°C	16 minutes, 37°C
OptiView HQ Linker	8 minutes (default)	8 minutes (default)	8 minutes (default)
OptiView HRP Multimer	8 minutes (default)	8 minutes (default)	8 minutes (default)
OptiView Amplification	Not Selected		
Counterstain	Hematoxylin II, 8 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".[14]

In addition to staining with VENTANA CLDN18 (43-14A) Assay, a second slide should be stained with Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001).

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. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in 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 control tissues for this antibody is gastric intestinal metaplasia, which demonstrates strong membranous staining in normal gastric epithelial cells and weak to moderate membranous staining of epithelial cells in the areas of metaplasia. Gastric intestinal metaplasia tissue has absence of CLDN18 staining in lamina propria, lymphocytes, smooth muscle, blood vessels, and peripheral nerve, which can be used as the negative control elements.

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by the VENTANA CLDN18 (43-14A) Assay. The cellular staining pattern for VENTANA CLDN18 (43-14A) Assay is membranous staining. Cytoplasmic staining can also be seen. A qualified pathologist experienced in immunohistochemical procedures must evaluate system level controls and qualify the stained slides before interpreting results.

SPECIFIC LIMITATIONS

VENTANA CLDN18 (43-14A) Assay will detect both CLDN18.1 and 18.2 proteins and may produce IHC staining in epithelial cells of normal gastric tissue[6,7] pneumocytes in normal lung tissue[6,7,12,15] and paneth cells of normal small intestine tissue.[5] CLDN18 expression within or in association with these cells have been reported in the published literature.[6,7,9-12,15,16]

This antibody has been optimized for specific incubation times (see Table 1) on the BenchMark IHC/ISH instrument in combination with the OptiView DAB IHC Detection Kit, but the user must validate results obtained with this reagent.

Studies support a minimum of 45 days of antigen stability on unstained slides. Slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate expiration dating within their own environment if dating beyond 45 days is desired.

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

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 2 and Table 3 and in the Precision section.

Sensitivity and Specificity

Table 2. Sensitivity/Specificity of VENTANA CLDN18 (43-14A) Assay was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Adrenal gland	0/3	Ovary (stroma)	0/3
Bladder	0/3	Parathyroid	0/3
Bone marrow	0/3	Pancreas	0/3
Breast	0/3	Peripheral nerve	0/3
Cerebellum	0/3	Prostate	0/3
Cerebrum	0/3	Salivary gland	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Colon	0/3	Skeletal muscle	0/3
Endometrium	0/3	Skin	0/3
Esophagus	0/3	Small intestine	2/3**
Heart	0/3	Spleen	0/3
Hypophysis	0/3	Stomach	12/12***
Kidney	0/3	Testis	0/3
Liver	0/3	Thymus	0/3
Lung	2/4*	Thyroid	0/3
Lymph node	0/3	Tonsil	0/3
Mesothelium	0/2	Uterine cervix	0/3

* Membrane CLDN18-staining observed in pneumocytes of 2/4 samples. No bronchial epithelium was present in 3/4 samples.

** Membrane and cytoplasmic CLDN18-staining observed in a sub-set of paneth cells.

*** Membrane and Cytoplasmic CLDN18 staining in gastric epithelium.

Table 3. Sensitivity/Specificity of VENTANA CLDN18 (43-14A) Assay was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Pancreatic neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Microinvasive ductal carcinoma (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
B-Cell Lymphoma; NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma ((Striated muscle)	0/1
Melanoma (Rectum)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	1/1*
B-Cell Lymphoma; NOS (Lymph node)	0/2
Hodgkin's lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Rhabdomyosarcoma (Peritoneum)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

* CLDN18-staining observed in approximately 3% of malignant cells

Precision

Precision studies for VENTANA CLDN18 (43-14A) Assay 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 instruments.
- Between platform precision between the BenchMark XT, BenchMark GX, BenchMark ULTRA instruments.

All studies met their acceptance criteria.

Method Comparison

Method comparison studies for VENTANA CLDN18 (43-14A) Assay were conducted between BenchMark XT, BenchMark GX, and BenchMark ULTRA instruments.

All studies met their acceptance criteria.

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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
C	Correct Number of tests to 50
B	Updates to Material Provided, Materials Required but not Provided, Warnings and Precautions, Symbols, Intellectual Property, and Contact Information

INTELLECTUAL PROPERTY

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