VENTANA®

Roche

anti-Glypican 3 (GC33) Mouse Monoclonal Primary Antibody

REF 790-4564

06483186001

IVD Σ 50

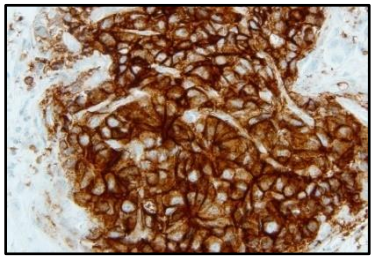

Figure 1. Anti-Glypican 3 (GC33) antibody staining hepatocellular carcinoma.

INTENDED USE

Anti-Glypican 3 (GC33) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of glypican 3 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

Glypican 3, a 70 kDa member of the glypican family, is encoded by the GPC3 gene located on the X chromosome (Xq26.2).[1,2] Glypican 3 has a structure consisting of a core protein and a heparan sulfate chain and binds to the cell membrane via a glycosylphosphatidylinositol anchor.[2] During embryogenesis, glypican 3 is expressed abundantly in multiple tissues and after birth, glypican 3 expression is decreased in healthy tissues.[3,4] Glypican 3 is anchored to the cell membrane and does not transmit intracellular signals due to the absence of a transmembrane domain.[1] Glypican 3 function appears to be primarily driven by its interactions in the extracellular matrix where it can recruit extracellular ligands and coordinate ligand-receptor interactions in the extracellular space.[1,5]

Hepatocytes are parenchymal cells of the liver and comprise roughly 80% of the total cell population of the liver.[6] Under normal physiological conditions, glypican 3 is generally not expressed in adult liver.[1,2] In contrast, glypican 3 expression has been detected in hepatocellular carcinoma, a primary liver cancer that originates from hepatocytes.[4,7] Immunohistochemistry (IHC) has been used to assess glypican 3 expression in hepatocellular carcinoma.[8,9,10] Several studies report glypican 3 expression in 70%-90% of hepatocellular carcinomas and the absence of glypican 3 in 100% of benign liver tissues.[8-11]

The detection of glypican 3 by immunohistochemistry (IHC) with anti-Glypican 3 (GC33) Mouse Monoclonal Primary Antibody (anti-Glypican 3 (GC33) antibody) may be used to aid in the differentiation of hepatocellular carcinoma from benign liver.

PRINCIPLE OF THE PROCEDURE

Anti-Glypican 3 (GC33) antibody binds to the glypican 3 protein 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 method sheet for more information.

MATERIAL PROVIDED

Anti-Glypican 3 (GC33) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-Glypican 3 (GC33) antibody contains approximately 7.5 µg of a mouse monoclonal antibody.

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

Specific antibody concentration is approximately 1.5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-Glypican 3 (GC33) antibody is a recombinant mouse monoclonal antibody produced as 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. General purpose laboratory equipment
15. 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 formalin-fixed, paraffin-embedded (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.[12] 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.

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. Do not use beyond the specified number of tests.
4. 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.
5. 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.
6. 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.[13,14]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.

9. 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 navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. 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 mist or vapours.
	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, a 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 Table 2 and Table 3 for recommended staining protocol.

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 790-4564.

Table 2. Recommended staining protocol for anti-Glypican 3 (GC33) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	ULTRA CC1, 36 minutes (Mild), 95 °C
Antibody (Primary)	16 minutes, 37 °C	32 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-Glypican 3 (GC33) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 32 minutes	ULTRA CC1, 32 minutes, 100 °C
Pre-Primary Peroxidase Inhibitor	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36 °C
OptiView HQ Linker	8 minutes	
OptiView HRP Multimer	8 minutes	
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."[15]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-Glypican 3 (GC33) 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. 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.

Examples of positive control tissues for this antibody are hepatocellular carcinoma and placenta.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for anti-Glypican 3 (GC33) antibody is cytoplasmic / membranous.

SPECIFIC LIMITATIONS

This antibody may demonstrate some weak to moderate diffuse granular cytoplasmic staining in the islet cells of the pancreas, parietal and chief cells of the stomach, enteroendocrine and/or Paneth cells of the colon, myocytes and myofibers in cardiac and skeletal muscle, respectively, and tubular epithelium of the kidney. Mild background staining or weak staining may be seen in endothelial cells.

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

OptiView detection system is generally more sensitive than *ultra*View Universal DAB Detection Kit. The user must validate the results obtained with this reagent and detection systems.

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-Glypican 3 (GC33) antibody was determined by testing FFPE non-neoplastic tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/4	Stomach	0/4
Cerebellum	0/4	Small intestine	0/4
Adrenal gland[a]	1/4	Colon[f]	1/4
Ovary	0/4	Rectum	0/1
Pancreas[b]	2/3	Liver	0/54
Lymph Node[c]	0/4	Liver cirrhosis[g]	1/17
Parathyroid gland	0/3	Liver hepatitis	0/7
Pituitary gland[d]	1/3	Salivary gland	0/4
Testis	0/4	Kidney[h]	2/4
Thyroid	0/4	Prostate[i]	0/4
Breast	0/3	Bladder	0/4
Spleen	0/3	Endometrium	0/3
Tonsil	0/3	Cervix	0/3
Thymus	0/3	Skeletal muscle[j]	2/3
Bone marrow	0/3	Skin	0/4
Lung	0/4	Nerve	0/3
Heart[e]	2/3	Mesothelium	0/3
Esophagus	0/4		

[a] Chromaffin cells

[b] Islet cells

[c] Tissues evaluated include normal and reactive.

[d] Rare anterior pituitary cells

[e] Myocytes

[f] Enteroendocrine cells and/or Paneth cells

[g] Few hepatocytes with weak staining

[h] Tubular epithelial cells

[i] Tissues evaluated include normal and hyperplasia.

[j] Myofibers

Table 5. Sensitivity/Specificity of anti-Glypican 3 (GC33) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Astrocytoma (Brain)	0/1
Meningioma (Cerebrum)	0/3
Adenocarcinoma (Head and neck)	0/1
Squamous cell carcinoma (Head and neck)	0/1
Nasopharyngeal carcinoma, NPC (Head and neck, nasopharynx)	0/1
Pleomorphic adenoma (Head and neck, salivary gland)	0/1
Adenoid cystic carcinoma (Head and neck, salivary gland)	0/1
Adenoma, cortical (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	0/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Endometrioid adenocarcinoma (Ovary)	0/1
Metastatic colon signet ring cell carcinoma (Ovary)	0/1
Seminoma (Testis)[a]	1/1
Adenoma (Thyroid)	0/2
Follicular carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Invasive ductal carcinoma (Breast)	0/3
Fibroadenoma (Breast)	0/2
Metastatic breast ductal carcinoma (Lymph node)	0/1
Small cell carcinoma (Lung)[b]	1/1
Squamous cell carcinoma (Lung)	0/2
Adenocarcinoma (Lung)	0/1
Metastatic cancer (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/2
Metastatic esophagus squamous cell carcinoma (Lymph node)	0/1
Adenocarcinoma (Stomach)	0/3
Adenocarcinoma (Small intestine)	0/1
Adenoma (Small intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/3
Metastatic colon adenocarcinoma (Liver)[b]	1/9
Adenocarcinoma (Rectum)[b]	1/3
Cholangiocarcinoma (Liver)	0/12
Hepatocellular carcinoma (Liver)	124/146
Metastatic hepatocellular carcinoma (Abdominal cavity)	0/1
Metastatic hepatocellular carcinoma (Pelvic cavity)	1/1
Metastatic hepatocellular carcinoma (Chest wall)	2/2
Metastatic hepatocellular carcinoma (Colon)	0/1
Neuroendocrine carcinoma (Liver)	0/2
Clear cell carcinoma (Kidney)	0/2
Adenocarcinoma (Prostate)	0/2
Urothelial carcinoma (Bladder)	0/2
Adenocarcinoma (Uterus)	0/2
Squamous cell carcinoma (Cervix)	0/2
B-cell lymphoma, NOS (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1

Pathology	# positive / total cases
Hodgkin lymphoma (Lymph node)	0/1
Squamous cell carcinoma (Skin)	0/1
Melanoma	0/1
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	1/1

[a] Rare tumor cells with very weak staining

[b] Some tumor cells with weak staining

Precision

Precision studies for anti-Glypican 3 (GC33) 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 and BenchMark ULTRA / ULTRA PLUS instrument.
- Between platform precision between the BenchMark GX and BenchMark ULTRA / ULTRA PLUS 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 Instrument Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of anti-Glypican 3 (GC33) 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 elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
F	Updates to the Warnings and Precautions, Sensitivity and Specificity, and Symbols sections.

INTELLECTUAL PROPERTY

For USA: Rx only

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