CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody

REF 790-4286

05278384001

IVD Σ 50

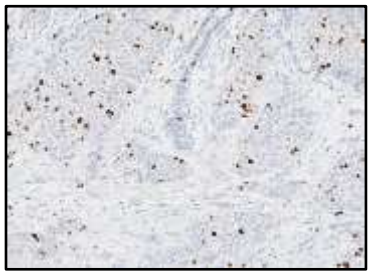

Figure 1. CONFIRM anti-Ki67 (30-9) antibody staining of breast carcinoma.

INTENDED USE

CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of Ki-67 protein 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

CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody (CONFIRM anti-Ki-67 (30-9) antibody) recognizes marker of proliferation Ki-67 (MKI67), more commonly known as Ki-67.

Ki-67 is a nuclear protein expressed in proliferating cells. Ki-67 encodes two protein isoforms with molecular weights of 345 and 395 kDa.[1] Ki-67 is present during all active phases of the cell cycle (G1, S, G2, and M), but is absent in resting cells (G0).[1] Ki-67 expression fluctuates during active cell cycle phases, beginning in G1 phase and increasing during S phase with peak levels occurring in metaphase.[2] During anaphase and telophase, Ki-67 expression begins to decrease.[2] The function of Ki-67 has not been fully elucidated, and little is known beyond it being a protein phosphorylated via serine and threonine with a critical role in cell division.[3]

The absence of Ki-67 in resting cells and its expression in all proliferating cells, whether normal or neoplastic, makes the Ki-67 antibody useful for determining the growth fraction of any given human cell population.[1]

Detection of Ki-67 protein by immunohistochemistry (IHC) with the CONFIRM anti-Ki-67 (30-9) antibody may be used to aid in the identification of cell proliferation in normal and neoplastic tissue. It may be used as part of a panel of IHC studies. The staining pattern is nuclear.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Ki-67 (30-9) antibody 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) that binds to the antigen, a secondary antibody (link antibody) that binds 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.

CONFIRM anti-Ki-67 (30-9) antibody is optimally diluted for use with *ultra*View Universal DAB and OptiView DAB IHC detection kits and BenchMark IHC/ISH instruments. For more detailed information on instrument operation, refer to the appropriate instrument User Guide.

MATERIAL PROVIDED

CONFIRM anti-Ki-67 (30-9) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-Ki-67 (30-9) antibody contains approximately 10 µg of a rabbit monoclonal antibody directed against Ki-67 present in tissue.

The antibody is diluted in Tris-HCl with carrier protein and 0.10% ProClin 300 a preservative.

Specific antibody concentration is approximately 2 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-Ki-67 (30-9) antibody is a rabbit 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. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
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. Mounting medium
15. Cover glass
16. General purpose laboratory equipment
17. 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 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.[4] 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.[5,6]
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.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P273	Avoid release to the environment.
	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 790-4286.

Table 2. Recommended staining protocols for CONFIRM anti-Ki-67 (30-9) 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, 64 minutes	CC1, 64 minutes	ULTRA CC1 64 minutes, 100°C
Pre Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	16 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.

Table 3. Recommended staining protocols for CONFIRM anti-Ki-67 (30-9) 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, Standard	CC1, Standard	ULTRA CC1 64 minutes, 95°C
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	16 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.

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."[7]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-Ki-67 (30-9) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

A tissue control must be included with each staining run. 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 tonsil or lymph node.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Ki-67 (30-9) antibody is nuclear.

SPECIFIC LIMITATIONS

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.

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 CONFIRM anti-Ki-67 (30-9) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	3/3	Thymus	3/3
Cerebellum	3/3	Bone marrow	3/3
Adrenal gland	1/3	Lung	2/3
Ovary	2/3	Heart	2/3
Pancreas	3/3	Esophagus	3/3
Lymph node	3/3	Stomach	3/3
Parathyroid gland	3/3	Small intestine	3/3
Pituitary gland	3/3	Colon	3/3
Testis	3/3	Liver	2/3
Thyroid	3/3	Salivary gland	2/3
Breast [a]	12/13	Kidney	2/3
Spleen	3/3	Prostate	1/3
Tonsil	3/3	Cervix	4/4
Endometrium	2/3	Skin	3/3
Skeletal muscle	0/3	Mesothelium	0/3
Nerve	0/3	Bladder	3/3

[a] Tissues evaluated include normal, hyperplasia and chronic inflammation.

Table 5. Sensitivity/Specificity of CONFIRM anti-Ki-67 (30-9) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	2/2
Meningioma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	1/1
Endometrioid carcinoma (Ovary)	1/1
Mucinous adenocarcinoma (Ovary)	1/1
Pancreatic neuroendocrine neoplasm (Pancreas)	1/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	1/1
Embryonal carcinoma (Testis)	1/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	1/1
Invasive ductal carcinoma (Breast)	120/124
Invasive lobular carcinoma (Breast)	5/5
Mucinous carcinoma (Breast)	1/1
Neuroendocrine carcinoma (Breast)	1/1
Medullary carcinoma (Breast)	7/7
Adenosis (Breast)	1/1
Breast carcinoma (Metastatic)	38/39
B-cell lymphoma; NOS (Spleen)	1/1
Squamous cell carcinoma (Lung)	1/1
Small cell carcinoma (Lung)	1/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Adenocarcinoma (Stomach)	1/1
Adenocarcinoma (Small intestine)	1/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	1/1
Adenocarcinoma (Colon)	1/1
GIST (Colon)	1/1
Adenocarcinoma (Rectum)	1/1
GIST (Rectum)	1/1
Melanoma (Rectum)	1/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	1/1
Clear cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Leiomyosarcoma (Uterus)	1/1
Adenocarcinoma (Uterus)	1/1

Pathology	# positive / total cases
Clear cell carcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	1/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Nerve)	1/1
Neuroblastoma (Retroperitoneum)	1/1
Pleomorphic rhabdomyosarcoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	1/1
B cell lymphoma, NOS (Lymph node)	2/2
Hodgkin's Lymphoma (Lymph node)	1/1
Anaplastic large cell lymphoma (Lymph node)	1/1
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma (Bladder)	1/1
Osteosarcoma (Cartilage)	0/1
Leiomyosarcoma (Smooth muscle)	1/1

Precision

Precision studies for CONFIRM anti-Ki-67 (30-9) 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 XT, BenchMark GX, 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.

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
F	Updates to Material Provided, Storage and Stability, Specimen Preparation, Warnings and Precautions, Staining Procedure, Positive Tissue Control, Specimen Preparation, Warnings and Precautions, Staining Procedure, Analytical Performance and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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