CONFIRM anti-Kappa Rabbit Polyclonal Primary Antibody

REF 760-2514

05267013001

IVD Σ 50

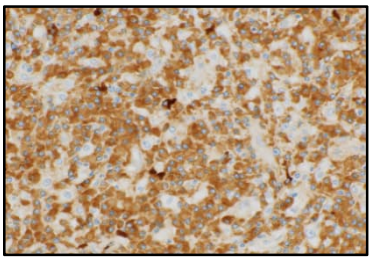

Figure 1. CONFIRM anti-Kappa antibody staining of a plasma cell neoplasm.

INTENDED USE

CONFIRM anti-Kappa Rabbit Polyclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of the Kappa light chain 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-Kappa Rabbit Polyclonal Primary Antibody (CONFIRM anti-Kappa antibody) detects Kappa light chain proteins. Kappa light chains are polypeptide chains, which in combination with heavy chains, form immunoglobulin molecules.[1,2,3] There are two classes of light chains found in immunoglobulins: Kappa light chains and Lambda light chains.[2,3] Light chain production by lymphoid cells is genetically restricted such that the immunoglobulin molecules produced by an individual cell will only contain a single light chain class (either Kappa or Lambda), but not both.[2,3] Plasma cell neoplasms often exhibit light chain restriction, in which the normal ratio of Kappa to Lambda immunoglobulin light chain expressing plasma cells is skewed.[3-6] Detection of clonal light chain restriction based on the Kappa to Lambda ratio is often used to differentiate monoclonal proliferations, which are often malignant, from polyclonal proliferations that arise during normal or reactive immune responses.[3-6]

CONFIRM anti-Kappa antibody may be used to detect Kappa light chain protein expression in plasma cells by immunohistochemistry (IHC). Determination of the ratio of Kappa to Lambda light chain expression in this cell population may be used to aid in the differentiation between a reactive process and a plasma cell neoplasm. This antibody may be used as part of a panel of IHC studies. The staining pattern is cytoplasmic and/or membraneous.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Kappa antibody binds to human Kappa light chain protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheets for further information.

MATERIAL PROVIDED

CONFIRM anti-Kappa antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-Kappa antibody contains approximately 33.5 µg of a rabbit polyclonal antibody.

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

Specific antibody concentration is approximately 6.7 µg/mL.

CONFIRM anti-Kappa antibody is a rabbit polyclonal antibody produced as rabbit serum.

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. CONFIRM Negative Control Rabbit Ig (Cat. No. 760-1029 / 05266238001)
4. ultraView Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
6. Protease 3 (Cat. No. 760-2020 / 05266718001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass
15. Automated coverslipper
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 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.[7] 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.[8,9]
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.
	H412	Harmful to aquatic life with long lasting effects
	P261	Avoid breathing mist or vapours.
	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 760-2514.

Table 2. Recommended staining protocol for CONFIRM anti-Kappa antibody with *ultra*View Universal DAB Detection Kit.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	None Selected	None Selected	None Selected
Enzyme	Protease 3, 16 minutes	Protease 3, 16 minutes	Protease 3, 16 minutes
Antibody (Primary)	8 minutes, 37°C	8 minutes, 37°C	8 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 CONFIRM anti-Kappa antibody with OptiView DAB IHC Detection Kit.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	None Selected	None Selected	None Selected
Enzyme	Protease 3, 16 minutes	Protease 3, 16 minutes	Protease 3, 16 minutes
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."[10]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-Kappa 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 the test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagent 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, lymph node, or spleen.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Kappa antibody is cytoplasmic and/or membranous.

SPECIFIC LIMITATIONS

Some degree of staining may be observed in the stroma and other tissue elements which is the result of secreted immunoglobulins and some non-specific background. This antibody does not show reliable staining in B cells, due to low sensitivity and high non-specific staining. Evaluation should be limited to plasma cells and neoplasms derived from plasma cells.

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

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

In addition to the evaluation of Kappa staining, Kappa and Lambda analytes were evaluated concurrently to assess for restriction status. Normal/reactive lymphoid tissue that stained positive with both are interpreted as non-restricted, B cell/plasma cell neoplasms that stained positive with either are interpreted as light chain restricted. If either analyte was not evaluable (no staining, high background, no tissue present), restriction status could not be determined.

Dense stromal staining is observed in many normal tissues. Plasma cells are also present in many normal non-lymphoid tissues. In those tissues with dense stromal staining or plasma cell staining, only the epithelium (or relevant organ cell type) is assessed for positive / negative status.

Table 4. Sensitivity/Specificity of CONFIRM anti-Kappa antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Stomach	0/5
Cerebellum	0/6	Small intestine	0/6
Cerebral cortex	0/3	Colon	0/6
Eye	0/2	Rectum	0/2
Adrenal gland	0/6	Appendix	0/3
Ovary	0/4	Liver	0/3
Pancreas	0/6	Salivary gland	0/4
Lymph node[a,b]	7/7	Kidney	0/5
Parathyroid gland	0/3	Prostate	0/6
Pituitary gland	0/4	Bladder	0/5
Testis	0/5	Ureter	0/2
Thyroid	0/6	Endometrium	0/5
Breast	0/6	Fallopian tube	0/3
Spleen[a]	8/8	Cervix	0/6
Tonsil[a,b]	87/87	Placenta	0/1
Thymus	0/6	Spinal cord	0/2
Bone marrow[a,c]	4/7	Skeletal muscle	0/6
Larynx	0/3	Skin	0/7
Lung[d]	0/5	Nerve	0/4
Heart	0/6	Mesothelium	0/3
Esophagus	0/6		

[a] Non-restricted [b] Normal and reactive tissue; [c] Three negative cases had indeterminate restriction status; [d] Normal and chronic infection tissue

Table 5. Sensitivity/Specificity of CONFIRM anti-Kappa antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Adenocarcinoma (Sinus)	0/1
Squamous cell carcinoma (Sinus)	0/1
Adenoma (Parathyroid Gland)	0/2
Adenoma (Adrenal gland)	0/1
Pheochromocytoma (Adrenal gland)	0/1
Adult granulosa cell tumor (Ovary)	0/1
Serous carcinoma (Ovary)	0/1
Teratoma (Ovary)	0/1
Neuroendocrine carcinoma (Pancreas)	0/1
Ductal adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/2
Follicular carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
Invasive lobular carcinoma (Breast)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Small cell carcinoma (Lung)	0/1
Myxoma (Heart)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach)	0/1
Gastrointestinal stromal tumor (Stomach)	0/1
Mesothelioma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (Small intestine)	0/1
Well-differentiated neuroendocrine tumor (Appendix)	0/1
Adenocarcinoma (Colon)	0/1
Adenosquamous carcinoma (Colon)	0/1
Cholangiocarcinoma (Liver)	0/1
Hepatocellular carcinoma (Liver)	0/1
Pleomorphic adenoma (Salivary gland)	0/1
Warthin tumor (Salivary gland)	0/1
Papillary adenoma (Kidney)	0/1
Renal cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Squamous cell carcinoma (Bladder)	0/1
Urothelial carcinoma (Bladder)	0/1
Clear cell carcinoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1

Pathology	# positive / total cases
Leiomyoma (Uterus)	0/1
Leiomyosarcoma (Uterus)	0/1
Adenocarcinoma (Cervix)	0/1
Squamous cell carcinoma (Cervix)	0/1
Basal cell carcinoma (Skin)	0/1
Melanoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Schwannoma (Peripheral nerve)	0/2
Schwannoma (Spinal cord)	0/1
Angiosarcoma (Soft tissue)	0/1
Alveolar rhabdomyosarcoma (Striated muscle)	0/1
Lymphoma, NOS[b]	0/7
Hodgkin lymphoma	0/12
B-cell lymphoma, NOS[b]	0/31
Diffuse large B-cell lymphoma[a,b]	2/7
MALT lymphoma[a,b]	1/7
Mantle cell lymphoma[b]	0/1
Follicular lymphoma[b]	0/1
Peripheral T-cell lymphoma	0/51
Anaplastic large cell lymphoma	0/10
NK/T-cell lymphoma	0/5
Multiple myeloma (Bone marrow)[a,b,c]	16/25

[a] Light chain restricted; [b] Some cases were unevaluable for Kappa or Lambda and restriction status was indeterminate; [c] 16/25 were Kappa restricted, 8/25 were Lambda restricted, 1/25 was negative for both

Precision

Precision studies for CONFIRM anti-Kappa 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, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT, BenchMark GX, 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 CONFIRM anti-Kappa 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
E	Updates to Warnings and Precautions section. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

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