CONFIRM anti-EMA (E29) Mouse Monoclonal Primary Antibody

REF 790-4463

05878900001

IVD Σ 50

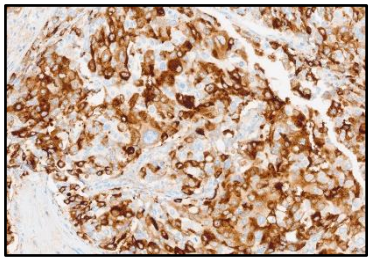

Figure 1. CONFIRM anti-EMA (E29) antibody staining breast invasive ductal carcinoma.

INTENDED USE

CONFIRM anti-EMA (E29) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of epithelial membrane antigen 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-EMA (E29) Mouse Monoclonal Primary Antibody (CONFIRM anti-EMA (E29) antibody) was raised against purified human milk fat globulin membrane preparation and specifically recognizes human epithelial membrane antigen (EMA).[1] EMA is a glycosylated transmembrane protein which lines the apical surface of epithelial cells in several organs and tissues including the lungs, stomach, pancreas, kidney, colon, and breast.[2-5]

EMA expression is characteristic of most carcinomas but is generally absent in lymphomas, leukemias, melanomas, and sarcomas.[4-7] There are some caveats: in particular, not all epithelial neoplasms are EMA positive: hepatocellular carcinomas, adrenocortical carcinomas, and malignant germ cell neoplasms exhibit little or no expression of EMA.[8] An IHC assay to detect EMA is useful as part of a panel of assays for the identification of carcinomas. The detection of EMA by IHC with CONFIRM anti-EMA (E29) antibody may be used to aid in the identification of tumors of epithelial origin.

EMA expression can be detected in 69-95% of mesotheliomas and may aid in the diagnosis of these tumors.[9-12] EMA may be useful in distinguishing between reactive mesothelial hyperplasia (typically EMA negative) and mesothelioma (typically EMA positive).[9,11] The detection of EMA by IHC with CONFIRM anti-EMA (E29) antibody may be used to aid in the diagnosis of mesothelioma.

Meningioma is one of the most common primary brain tumors and the majority of these lesions are slow growing and benign.[13] EMA expression has been detected in 90-100% cases of meningioma.[14-17] IHC assays specific for EMA can be used to aid in the diagnosis these tumors.[14,18,19] In the case of a differential diagnosis, the use of EMA in combination with other markers specific to meningioma as well as markers specific for the confounding tumor(s) should be used to confirm the diagnosis.[14,18,19] The detection of EMA by IHC with CONFIRM anti-EMA (E29) antibody may be used to aid in the diagnosis of meningioma.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-EMA (E29) antibody binds to the EMA 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 the OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-EMA (E29) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-EMA (E29) antibody contains approximately 1.5 µg of a mouse monoclonal antibody.

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

Specific antibody concentration is approximately 0.3 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-EMA (E29) antibody is a recombinant mouse monoclonal antibody produced from purified 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.[20] 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.[21,22]
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, 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 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 790-4463.

Table 2. Recommended staining protocol for CONFIRM anti-EMA (E29) 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 Standard	ULTRA CC1 64 minutes, 95 °C
Antibody (Primary)	20 minutes, 37 °C	20 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-EMA (E29) 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)	12 minutes, 37 °C	12 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."[23]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-EMA (E29) 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.

An example of positive control tissues for this antibody is normal pancreas.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-EMA (E29) antibody is membranous and / or cytoplasmic.

SPECIFIC LIMITATIONS

OptiView detection system is generally more sensitive than the *ultra*View detection system. 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-EMA (E29) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/4	Stomach[j]	4/4
Cerebellum	0/4	Small intestine[j]	4/4
Eye	0/2	Colon[j]	3/3
Adrenal gland[a]	0/4	Rectum[j]	3/4
Ovary	0/4	Liver	0/4
Pancreas[b,c]	3/3	Salivary gland[b]	3/3
Lymph node[d,e]	1/1	Kidney[k,l,m]	14/15
Parathyroid gland	0/3	Prostate[a,j]	3/4
Pituitary gland	0/3	Bladder[n]	3/3
Testis	0/4	Ureter[n]	1/1
Thyroid	0/4	Endometrium[j]	3/3
Breast[a,b,f]	5/5	Fallopian tube[j]	2/2
Spleen	0/3	Placenta[o]	1/3
Tonsil[g]	2/3	Cervix[i]	3/4
Thymus[g]	3/3	Skeletal muscle	0/3
Bone marrow	0/3	Skin[p]	2/3
Lung[h]	4/4	Nerve	0/5
Heart	0/3	Spinal cord	0/2
Esophagus[i]	4/4	Mesothelium	2/3

EMA is expressed on the apical surface of epithelial cells in several organs and tissues and may stain many normal structures such as those listed below:

[a] Tissue evaluated includes normal and hyperplasia; [b] Ductal cells; [c] Acinar cells; [d] Tissue evaluated includes normal and reactive; [e] Reactive immune cells; [f] Lobular cells; [g] Squamous cells; [h] Pneumocytes; [i] Squamous epithelial cell; [j] Glandular cells; [k] Tissue evaluated includes normal, atrophied, and inflammatory; [l] Tubules; [m] Minor specific off target staining in lymphocytes; [n] Urothelium cells; [o] Trophoblasts; [p] Sebaceous glandular cells.

Table 5. Sensitivity/Specificity of CONFIRM anti-EMA (E29) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Meningioma (Cerebellum)[a]	73/82
Meningioma (Cerebrum)	0/1
Astrocytoma (Brain)	1/1
Squamous cell carcinoma (Head and neck)	1/1
Nasopharyngeal carcinoma (Nasopharynx)	1/1
Adenoma (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	0/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	2/2
Metastatic colon signet ring cell carcinoma (Ovary)	1/1
Metastatic breast ductal carcinoma (Lymph node)	1/1
Metastatic esophagus squamous cell carcinoma (Lymph Node)	1/1
Adenoma (Parathyroid)	0/2
Seminoma (Testis)	0/2
Adenoma (Thyroid)	0/3
Follicular carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Fibroadenoma (Breast)	1/2
Invasive carcinoma of no special type (Breast)	53/54
Ductal carcinoma in situ (Breast)	5/5
Invasive lobular carcinoma (Breast)	3/3
Invasive micropapillary carcinoma (Breast)	1/1
Squamous cell carcinoma (Lung)	0/2
Adenocarcinoma (Lung)	1/1
Small cell carcinoma (Lung)	0/1
Adenocarcinoma (Stomach)	3/3
Adenoma (Small intestine)	1/1
Adenocarcinoma (Small intestine)	1/1
Adenoma (Colon)	1/1
Adenocarcinoma (Colon)	3/3
Metastatic gastrointestinal carcinoma (Lung)	0/1
Metastatic colon adenocarcinoma (Liver)	1/1
Adenocarcinoma (Rectum)	3/3
Hepatocellular carcinoma (Liver)	1/4
Pleomorphic adenoma (Salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	1/1
Angioleiomyoma (Kidney)	0/1
Clear cell carcinoma (Kidney)	20/20
Papillary cell carcinoma (Kidney)	6/7
Chromophobe renal cell carcinoma (Kidney)	3/3
Urothelial Carcinoma (Kidney)	2/2
Squamous cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	0/2
Urothelial carcinoma (Bladder)	2/2
Adenocarcinoma (Endometrium)	2/2
Squamous cell carcinoma (Cervix)	2/2

Pathology	# positive / total cases
Squamous cell carcinoma (Skin)	1/1
Mesothelioma[b]	33/44
Melanoma	0/1
Hodgkin lymphoma	0/1
B-cell lymphoma, NOS	0/2
Anaplastic large cell lymphoma	0/1
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	0/1
Adenocarcinoma (Bone)	0/1

[a] Tissue cases assessed consist meningioma of the following types: meningothelial, fibrous, transitional, malignant, psammomatous, angiomatious, microcystic, chordoid, or papillary

[b] Tissue cases assessed consist of epithelioid mesothelioma, sarcomatoid malignant mesothelioma, diffuse mesothelioma and mixed malignant mesothelioma sourced from sites that include but are not limited to peritoneum, retroperitoneum, omentum, pleura, pericardium, colon and kidney.

Precision

Precision studies for CONFIRM anti-EMA (E29) 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 Run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of CONFIRM anti-EMA (E29) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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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
C	Updates to Principle of Procedure, Material Provided, Materials Required But Not Provided, Warnings and Precautions, Staining Procedure, Analytical Performance, Precision and Intellectual Property sections. Removed XT, added GX, added OptiView DAB and updated Sensitivity and Specificity tables.

INTELLECTUAL PROPERTY

For USA: Rx only

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