# CONFIRM anti-EMA (E29) Mouse Monoclonal Primary Antibody

REF 790-4463

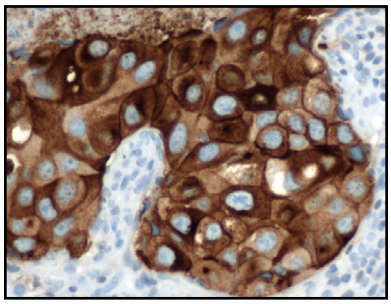

Figure 1. CONFIRM anti-EMA (E29) staining breast invasive ductal carcinoma

## INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-EMA (E29) Mouse Monoclonal Primary Antibody (CONFIRM anti-EMA (E29)) is directed against epithelial membrane antigen expressed on the apical surface of glandular epithelia. This glycoprotein is primarily membrane localized on normal and tumor tissue, although it may exhibit a cytoplasmic localization in less differentiated carcinomas. This antibody may be used to aid in the identification of epithelial membrane antigen in adenocarcinomas derived from secretory epithelia, malignant mesothelioma, renal cell carcinomas and meningiomas. The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

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) 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 is present on a variety of glandular epithelia such as the breast, eccrine and apocrine glands, and the pancreas,[2,3] and to a lesser degree on squamous epithelium. Gastrointestinal epithelium, endocervical epithelium and prostate glands exhibit little or no expression of EMA.[2] EMA is a useful marker as part of the panel of antibodies for the identification of carcinomas of epithelial origin. EMA expression may aid in distinguishing malignant mesothelioma from adenocarcinomas,[4] and assist in the identification of renal cell carcinoma and meningioma.[5,6] Detection of EMA may also be valuable in the detection of breast carcinoma metastases.[2,7] Most lymphomas, melanomas and sarcomas are negative.

## REAGENT PROVIDED

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

One 5 mL dispenser of CONFIRM anti-EMA (E29) contains approximately 2.7 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.05 M Tris HCl with 1% carrier protein and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.54 µg/mL. There is no known non-specific antibody reactivity observed in this product.

This antibody is optimized for use on a Ventana automated slide stainer in combination with Ventana detection kits. No reconstitution, mixing, dilution, or titration is required.

CONFIRM anti-EMA (E29) is produced as culture supernatant.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

## MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (i.e., *ultra*View Universal DAB Detection Kit), and ancillary components, including negative and positive tissue control slides, are not provided.

## STORAGE

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 tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[8] 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 use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local and/or state authorities with regard to recommended method of disposal.

## STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT/BenchMark ULTRA instruments with *ultra*View Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's 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 CONFIRM anti-EMA (E29) with *ultra*View Universal DAB Detection Kit on a BenchMark XT/BenchMark ULTRA instrument.

| Procedure Type | Method |
|---|---|
| Deparaffinization | Selected |
| Cell Conditioning (Antigen Unmasking) | Standard Cell Conditioning 1 |
| Enzyme (Protease) | None required |
| Antibody (Primary) | BenchMark XT instrument<br>Approximately 16 minutes, 37°C<br>BenchMark ULTRA instrument<br>Approximately 20 minutes, 36°C |
| Counterstain | Hematoxylin II, 4 minutes |
| Post Counterstain | Bluing Reagent, 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".[9]

## POSITIVE TISSUE CONTROL

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

## STAINING INTERPRETATION

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

## SPECIFIC LIMITATIONS

This antibody has been optimized on Ventana BenchMark XT and BenchMark ULTRA instruments in combination with *ultra*View Universal DAB Detection Kit (REF 760-500) at a 16 minute or 20 minute primary antibody incubation time, respectively. However, the user must validate individual laboratory results obtained with this reagent.

## PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-EMA (E29) was determined by testing formalin-fixed, paraffin-embedded normal and neoplastic tissues.

   For normal tissues, results are as follows: (0/3) cerebrum, (0/3) cerebellum, (0/3) adrenal gland, (0/3) ovary, (3/3) pancreas, (0/3) parathyroid, (0/3) hypophysis, (0/3) testis, (0/3) thyroid gland, (2/3) breast, (0/3) spleen, (3/3) tonsil, (2/3) thymus, (1/3) pulmonary, (0/3) myocardium, (2/3) esophagus, (1/3) small intestine, (3/3) colon, (0/3) liver, (3/3) salivary gland, (2/3) kidney, (0/3) prostate, and (3/3) lung.

   For neoplastic tissues, results are as follows: (53/60) cerebral meningioma, (0/1) malignant ependymoma, (0/1) malignant oligodendroglioma, (4/4) granular cell carcinoma of kidney, (5/5) clear cell carcinoma of kidney, (1/4) papillary renal cell carcinoma, (2/2) medullary carcinoma of kidney, (2/2) transitional cell carcinoma of kidney, (1/1) nephroblastoma of kidney, (2/2) squamous cell carcinoma of kidney, (28/38) malignant mesothelioma, (38/39) breast invasive ductal carcinoma, (1/1) lobular carcinoma in situ of breast, (2/3) intraductal carcinoma of breast, (0/1) skin malignant melanoma, (1/1) mucinous carcinoma, (0/1) glioblastoma, (2/2) papillary adenocarcinoma, (0/1) islet cell carcinoma, (1/1) pancreatic adenocarcinoma, (0/1) seminoma, (0/1) embryonal carcinoma of testis, (0/1) medullary carcinoma of thyroid, (1/1) papillary carcinoma of thyroid, (0/1) diffuse B-cell lymphoma, (1/1) squamous cell carcinoma of lung, (1/1) esophagial adenocarcinoma, (1/1) mucinous adenocarcinoma of stomach, (1/1) colon adenocarcinoma, (0/3) intermediate grade interstitialoma, (1/1) rectum adenocarcinoma, (1/1) hepatocellular carcinoma, (0/1) hepatoblastoma, (1/1) prostate adenocarcinoma, (1/1) transitional cell carcinoma of prostate, (0/1) leiomyoma, (1/1) endometrial adenocarcinoma, (1/1) Clear cell carcinoma of endometrium, (2/2) squamous cell carcinoma of cervix, (0/1) embryonal rhabdomyosarcoma of left leg, (0/1) malignant melanoma of anus, (0/1) basal cell carcinoma of head, (0/1) squamous cell carcinoma of left chest wall, (0/1) neuroblastoma of retroperitoneum, (0/2) diffuse malignant lymphoma, (0/1) Hodgkin's lymphoma of supraclavicular, (1/1) diffuse malignant lymphoma of mandible, (1/1) transitional cell carcinoma with squamous metaplasia of bladder, (0/1) low grade leiomyosarcoma of bladder, (0/1) osteosarcoma of right femur, (0/1) spindle cell rhabdomyosarcoma of retroperitoneum and (0/1) intermediate grade leiomyosarcoma.
2. Inter-lot reproducibility was determined by testing 3 lots across 3 tissue types on a BenchMark XT instrument. 18 out of 18 tested across all 3 lots scored equivalently.
3. Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on a BenchMark XT instrument over a five day non-consecutive period. 150 out of 150 samples tested scored equivalently.
4. Intra-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 14 slides on a BenchMark XT instrument. 84 out of 84 samples tested scored equivalently.
5. Intra-platform reproducibility was determined by staining 2 multi-tissue blocks (3 tissues each for a total of 6 tissues) across 5 slides on 3 BenchMark XT instruments. 90 out of 90 samples tested scored equivalently.
6. Intra-platform reproducibility was also determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 samples tested scored equivalently.
7. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 90 out of 90 samples tested scored equivalently
8. Compatible with *i*VIEW DAB and *ultra*View Universal DAB Detection Kits.

## REFERENCES


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## INTELLECTUAL PROPERTY



## CONTACT INFORMATION


Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.ventanamed.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany


CE