Ep-CAM/Epithelial Specific Antigen (Ber-EP4) Mouse Monoclonal Antibody

For In Vitro Diagnostic Use (IVD)

Product Identification

Ventana REF	Roche #	Description
760-4383	05435676001	50 test dispenser

Symbol Definitions

KEY-CODE	keycode
A	ascites
E	serum
S	supernatant

Intended Use

Ep-CAM/Epithelial Specific Antigen (Ber-EP4) Mouse Monoclonal Primary Antibody is intended for laboratory use in the detection of the Ep-CAM glycoprotein in formalin-fixed, paraffin-embedded human tissue stained in qualitative immunohistochemistry (IHC) on BenchMark IHC/ISH instruments. 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

Epithelial cell adhesion molecule (Ep-CAM) is a transmembrane glycoprotein localized on the membrane of cells in most epithelial tissues.[1] Immunoreactivity with the antibody to Ep-CAM has been seen in the majority of epithelial neoplasms, whereas most non-epithelial neoplasms do not show Ep-CAM expression.[2] Ep-CAM is not expressed in mesothelial cells, hepatocytes, and lymphocytes.[1,2] In conjunction with other markers, Ep-CAM can be used as an aid in determining epitheilial origin of neoplasms such as lung adenocarcinoma.[2-4]

Principles and Procedures

Ep-CAM/Epithelial Specific Antigen (Ber-EP4) Mouse Monoclonal Antibody (this antibody) may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical staining allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) 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 a coverslip applied. 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.

This antibody is optimally diluted to be compatible with VENTANA detection kits and BenchMark IHC/ISH instruments. Refer to the Tables in the Instructions for Use section for recommended staining protocols. Each step in the staining protocol includes incubation for a precise time at a specific temperature. At the end of each incubation step, the sections are rinsed by the BenchMark IHC/ISH instruments to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps. To minimize evaporation of the aqueous reagents from the specimen-containing slide, a coverslip solution is applied in the slide stainer. For more detailed information on instrument operation, refer to the appropriate BenchMark IHC/ISH instruments Operator's Manual.

Materials and Methods

Reagents Provided

One dispenser of this antibody contains sufficient prediluted reagent for 50 tests.

Product Composition	
Predilute: diluted in	Tris Buffer, pH 7.3-7.7, with 1% BSA and <0.1% Sodium Azide
Host	Mouse
Isotype	IgG_1/k
Source	Supernatant

See product label for lot specific information for the following:

1. Antibody immunoglobulin concentration
2. Source details

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. No reconstitution, mixing, dilution, or titration is required. Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls. (See Quality Control Procedures Section)

Materials and Reagents Needed But Not Provided

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and postive tissue control slides are not provided. Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

Storage and Handling

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.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Contact Cell Marque technical support if there is a suspected indication of reagent instability.

Specimen Collection and Preparation for Analysis

Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded, tissues are suitable for use with this antibody when used with VENTANA detection kit and accessories and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral-buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (approximately 4 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but no longer than 24 hours) in a 53-65°C oven.

Warnings and Precautions

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (example: xylene).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. Incubation times and temperatures other than those specified may give erroneous results.
6. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change.
7. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is less than 0.1% sodium azide and does not meet the OSHA (USA) criteria for hazardous substance at the stated concentration. See SDS.
8. The user must validate any storage conditions other than those specified in the package insert.
9. Diluent may contain bovine serum albumin and supernatant may contain bovine serum. The products containing fetal bovine serum and products containing bovine serum albumin are purchased from commercial suppliers. Certificates of Origin for the animal source used in these products are on file at Cell Marque. The certificates support that the bovine sources are from countries with negligible BSE risk and state sources of bovine from USA and Canada.
10. As with any product derived from biological sources, proper handling procedures should be used.

Instructions For Use

Step by Step Procedure

This antibody has been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories.

Recommended Staining Protocols:

Recommended staining protocol for this antibody with ultraView Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Recommended staining protocol with *ultra*View	
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	Not required
Antibody (Primary)	BenchMark ULTRA or ULTRA PLUS instrument: 16 minutes, 36°C BenchMark XT instrument: 16 minutes, 37°C BenchMark GX instrument: 16 minutes, 37°C
Amplification	Not selected
Counterstain	Hematoxylin II, 8 minutes
Post Counterstain	Bluing, 4 minutes

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Use of a tissue section fixed or processed differently from the test specimen will serve to provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. Positive tissue control for the stated primary antibody may include the following:

Positive Tissue Control	
Tissue	Visualization
Adenocarcinoma	Cytoplasmic, Membranous

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Negative Tissue Control

The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should demonstrate

the absence of specific staining, and provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

Unexplained Discrepancies

Unexplained discrepencies in controls should be referred to your local representative immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the entire procedure with the patient samples.

Negative Control Reagent

A negative control reagent must be run for every specimen to aid in the interpretation of results. A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. The slide should be treated with negative control reagent, matching the host species of the primary antibody, and ideally having the same IgG concentration. The incubation period for the negative control reagent should equal the primary antibody incubation period.

Interpretation of Results

The immunostaining procedure run on BenchMark IHC/ISH instruments causes a colored reaction product to precipitate at the antigen site localized by this antibody. Refer to the appropriate detection system package insert for expected color reactions. A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting results.

Positive Tissue Control

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Refer to the package insert of the detection system used for expected color reactions. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results. If the positive tissue control fails to demonstrate appropriate positive staining, any results with the test specimens are considered invalid.

Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen are considered invalid. Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from tissues that are not optimally fixed. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells show non-specific staining.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may aid in the identification of false negative reactions (see Summary of Expected Results section). The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

Limitations

1. Color does not affect performance.
2. This reagent is "for professional use only" as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation, processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
3. For laboratory use only.
4. For *in vitro* diagnostic use.
5. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the tissue.
6. Excessive or incomplete counterstaining may compromise proper interpretation of results.
7. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies if applicable. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
8. Cell Marque provides antibodies at optimal dilution for use as instructed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for interpretation of patient results.
9. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Cell Marque technical support with documented unexpected reactions.
10. This product is not intended for use in flow cytometry; performance characteristics have not been determined.
11. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
12. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
13. False positive results may be seen because of non immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) subject to the type of immunostaining technique used.
14. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

15. This antibody is optimized for the incubation time specified in the Instructions for Use section in combination with VENTANA detection kits and accessories and the BenchMark IHC/ISH instruments. Because of variation in the tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens.
16. This antibody, when used in combination with the VENTANA detection kits and accessories, detects antigen(s) that survive routine formalin-fixed, tissue processing, and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

Summary of Expected Results

See the following tables of reactivity:

Normal Study			
Tissue	# Stained (+)	Total #	Notes
Brain Cerebrum	0	4	
Brain Cerebellum	0	3	
Adrenal Gland	0	3	
Ovary	0	3	
Pancreas	3	3	Ducts +; acinar cells +; islet cells +
Parathyroid	3	3	Parenchymal cells +
Pituitary	3	3	Adenohypophysis +
Testis	0	3	
Thyroid	3	3	Follicular epithelium +
Breast	3	4	Ducts +; 1/3 lobules +
Spleen	0	3	
Tonsil	0	3	
Thymus	0	3	
Bone Marrow	0	3	
Lung	2	3	Bronchiolar and alveolar epithelial cells +; 2/2 bronchial epithelium +
Heart	0	3	
Esophagus	0	3	
Stomach	2	3	Mucosal epithelium +
Small Intestine	3	4	3/3 Mucosal epithelium +
Colon	5	5	Mucosal epithelium +
Liver	3	3	Bile ducts +
Salivary Gland	3	3	Duct cells +; acini +

Normal Study			
Tissue	# Stained (+)	Total #	Notes
Kidney	4	4	Distal tubules +; collecting ducts +
Prostate	2	3	Epithelium +
Uterus	3	3	Endometrial epithelium +
Cervix	3	3	Endocervical glands +
Skeletal Muscle	0	3	
Skin	3	3	Sweat glands+; 1/1 hair follicle +
Peripheral Nerve	0	3	
Mesothelial Lining	0	3	
Lymph Node	0	1	

Disease Tissue Study			
Tissue	# Stained (+)	Total #	Notes
Colorectal adenocarcinoma	19	19	
Breast invasive ductal carcinoma	14	14	
Lung adenocarcinoma	13	14	
Basal cell carcinoma	12	12	Diffuse
Chromophobe renal cell carcinoma	12	12	Diffuse
Clear cell renal cell carcinoma	3	14	Weak focal; weak rare
Skin squamous cell carcinoma	3	12	Weak to moderate, focal
Merkel cell carcinoma	1	1	
Mesothelioma	0	14	
Seborrheic keratosis	0	1	

Troubleshooting

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same staining run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label. If the slide is labeled properly, other positive controls used on the same instrument run should be checked to determine if it is because of the primary

antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.

3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included unless a biotin-free detection system is being used in which case any biotin present would not be a contributing factor to background staining.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific antibody staining is too intense, the run should be repeated with incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
6. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged. Other possibilities that could have adverse affect on tissue adhesion include insufficient drying of the tissue section on the slide prior to staining or fixation in formalin that was not properly neutral-buffered. Tissue thickness may also be a contributing factor.

For corrective action, refer to the Instructions for Use section or contact Cell Marque technical support at techsupport@cellmarque.com.

References

1. Schnell, Ulrike et al. "EpCAM: structure and function in health and disease." *Biochimica et biophysica acta* vol. 1828,8 (2013): 1989-2001.
2. Latza, U et al. "Ber-EP4: new monoclonal antibody which distinguishes epithelia from mesothelial." *Journal of clinical pathology* vol. 43,3 (1990): 213-9.
3. Ordóñez, N G. "Value of the Ber-EP4 antibody in differentiating epithelial pleural mesothelioma from adenocarcinoma. The M.D. Anderson experience and a critical review of the literature." *American journal of clinical pathology* vol. 109,1 (1998): 85-9.
4. Ordóñez, Nelson G. "The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma." *The American journal of surgical pathology* vol. 27,8 (2003): 1031-51.

Disclaimers

*VENTANA, ULTRAVIEW, OPTIVIEW, and BENCHMARK are registered trademarks of Ventana Medical Systems, Inc. Cell Marque™ antibodies are developed manufactured and distributed by Cell Marque™ and their sale through Ventana Medical Systems, Inc. (a member of the Roche Group) does not imply approval, endorsement, or any guarantee of quality or performance of those Cell Marque™ antibodies by Ventana Medical Systems, Inc.

www.cellmarque.com

Cell Marque Corporation
6600 Sierra College Blvd. • Rocklin, CA 95677 USA • 916-746-8900

EC REP EMERGO EUROPE
Prinsessegracht 20, 2514 AP The Hague, The Netherlands

CE

CM Template #4.1v1
Implemention date 10 Mar 2022