

CD47 [EPR21794]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3284-062822

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Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3284 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	API 3284 AA,	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3284 T60	60 tests	Ready-to-use	N/A
Q Series-- For Leica BOND-III	ALI 3284 G7	7.0 mL	Ready-to-use	N/A
UltraLine -- For BenchMark	AVI 3284 G	6.0 mL	Ready-to-use	N/A

Intended Use:

For *in vitro* Diagnostic Use

CD47 [EPR21794] is a rabbit monoclonal antibody that is intended for laboratory use after the initial diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains, in the qualitative identification of CD47 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist as an aid in making any other clinical determinations.

Summary and Explanation:

CD47 has been shown to have a role in both cell adhesion by acting as an adhesion receptor for THBS1 on platelets, and in the modulation of integrins.¹⁵ In the brain, it appears to play an important role in memory formation and synaptic plasticity in the hippocampus.¹⁶ CD47 is a receptor for SIRPA, binding to which prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells.¹⁷ Interaction with SIRPG mediates cell-cell adhesion, enhances superantigen-dependent T-cell-mediated proliferation and costimulates T-cell activation.¹⁵ CD47 may play a role in membrane transport and/or integrin dependent signal transduction. CD47 has been described as broadly expressed in many human tissues, with abundant expression in epithelial cells, the brain, and in ovarian cancer.¹⁸

Principle of Procedure:

This antibody product may be used as the primary antibody in immunohistochemistry testing of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (optional link antibody/probe), 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.

Materials and Methods:

Reagents Provided:

Host Source: Rabbit monoclonal

Species Reactivity: Human; other species not tested

Clone: [EPR21794]

Isotype: IgG

Protein Concentration: See vial label for lot specific concentration

Specific IgG Concentration: Contact Biocare's Technical Support

Specificity: CD47

Cellular Localization: Cell membrane

Method: Affinity purified recombinant rabbit monoclonal

Reconstitution, Mixing, Dilution, Titration:

Prediluted antibody reagent is optimally diluted for use with the above listed staining systems. Further dilution may result in loss of antigen staining. The user must validate any such change. Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results necessitating regular performance of in-house controls (see Quality Control section).

Concentrated reagent requires dilution as indicated in table above.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffered saline solution, pH 7.2 – 7.4, containing a protein carrier and <0.1% sodium azide preservative. See Safety Data Sheet for additional details.

Materials and Reagents Needed But Not Provided:

Microscope slides, positively charged
Positive and negative tissue controls
Desert Chamber (or similar Drying oven)
Xylene or xylene substitute
Ethanol or reagent alcohol
Decloaking Chamber (Pressure cooker)
Deionized or distilled water
Wash buffer
Pretreatment reagents
Peroxidase block
Protein block (optional)
Detection probe and polymer
Negative control reagents
Chromogens
Hematoxylin (counterstain)
Bluing reagent
Mounting medium
Coverglass
Light Microscope (40-400X magnification)

Configurations of the antibody product are available for use on the instruments indicated in the table above.

Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the vial label, when stored under these conditions. Do not use after expiration date. Storage under any condition other than those specified must be verified. Diluted reagents should be used promptly; store any remaining reagent at 2°C to 8°C. The stability of user diluted reagent has not been established by Biocare.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Biocare's Technical Support at 1-800-542-2002 or via the technical support information provided on biocare.net.

 Biocare Medical

60 Berry Drive
Pacheco, CA 94553

USA



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Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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Specimen Preparation:

Tissues fixed in formalin are suitable for use prior to paraffin embedding. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.^{1,2}

Properly fixed and embedded tissues expressing the specified antigen target should be stored in a cool place. The Clinical Laboratory Improvement Act (CLIA) of 1988 requires in 42 CFR §493.1259(b) that "The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination."³

Treatment of Tissues Prior to Staining:

Perform Heat Induced Epitope Retrieval (HIER) per recommended protocol below. The routine use of HIER prior to IHC has been shown to minimize inconsistency and standardize staining.^{4,5}

Warning and Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)⁶
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water.⁷
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. Prediluted antibody reagent is optimally diluted for use. Further dilution may result in loss of antigen staining.
7. Dilution of concentrated antibody reagent must be validated before use. Any diluent used that is not specifically recommended also must be validated for compatibility and stability.
8. The SDS is available upon request and is located at <http://biocare.net>.

Instructions for Use:

Recommended Staining Protocols for CD47 [EPR21794]:

IntelliPATH FLX and manual use:

API3284 for IntelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS	
Peroxide Block:	Block for 5 minutes with Peroxidized 1.
Pretreatment:	Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
Protein Block (Optional):	Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody:	Incubate for 30 minutes at RT.
Detection:	Probe: N/A Incubate for 0 minutes at RT with a secondary polymer.
	Polymer: Incubate for 30 minutes at RT with a tertiary polymer.

Chromogen:	Incubate for 5 minutes at RT with Biocare's DAB – OR –Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:	Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

ONCORE Pro Automated Slide Staining System:

OPAI3284 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:	
Protocol Name:	CD47 Rb
Protocol Template (Description):	Rb HRP Template 1
Dewaxing (DS Buffer Option):	DS2-50
Antigen Retrieval (AR Option):	AR1, high pH; 103°C
Block Option:	Buffer
Reagent Name, Time, Temp.:	CD47 Rb, 30 min., 25°C

Ventana BenchMark ULTRA:

AVI3284 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:	
Template/Detection:	OptiView DAB IHC
Pretreatment Protocol:	CC1 64 minutes
Peroxidase:	Pre-Primary Peroxidase Inhibitor
Primary Antibody:	48 minutes, 36°C

Q Series – For Leica BOND-III:

ALI3284 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:	
Chromogen Staining Option	DAB
Protocol Name:	IHC Protocol F
Detection:	Bond Polymer Refine
HIER:	30 min with ER2
Peroxide Block:	5 min
Marker (Primary Antibody):	15 min
Post Primary:	8 min
Polymer:	8 min
Mixed Chromogen Refine:	10 min
Hematoxylin:	5 min

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011⁸

Positive Tissue Control: Liver cancer, placenta

External Positive control materials should be fresh specimens fixed, processed, and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive external tissue control for each set of test conditions should be included in each staining run.

The tissues used for the external positive control materials should be selected from patient specimens with well-characterized low levels of the positive

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target activity that gives weak positive staining. The low level of positivity for external positive controls is designed so to ensure detection of subtle changes in the primary antibody sensitivity from instability or problems with the IHC methodology. Commercially available tissue control slides or specimens processed differently from the patient sample(s) validate reagent performance only and do not verify tissue preparation.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control:

Use a negative tissue control (known to be CD47 negative) fixed, processed, and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the IHC primary antibody for demonstration of the target antigen, and to provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can be used by the laboratorian as internal negative control sites to verify the IHC's performance specifications. The types and sources of specimens that may be used for negative tissue controls are listed in the Performance Characteristics section.

If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Nonspecific Negative Reagent Control:

Use a nonspecific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. Ideally, a negative reagent control contains a CD47 IgG antibody produced from tissue culture supernatant in the same way as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix/solution as the Biocare antibody. Dilute a negative control antibody to the same immunoglobulin or protein concentration as the diluted primary antibody using the identical diluent. If fetal calf serum is retained in the neat antibody after processing, fetal calf serum at a protein concentration equivalent to the diluted primary antibody in the same diluent is also suitable for use. (Refer to reagent provided). Diluent alone may be used as a less desirable alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should correspond to that of the primary antibody.

When panels of several antibodies are used on serial sections, the negatively staining areas of one slide may serve as a negative/nonspecific binding background control for other antibodies. To differentiate endogenous enzyme activity or nonspecific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate-chromogen or enzyme complexes (PAP, avidin-biotin, streptavidin) and substrate-chromogen, respectively.

Assay Verification:

Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody's specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative tissues. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP Certification Program⁹ for Immunohistochemistry and/or the NCCLS IHC guideline¹⁰). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Tissues listed in the Performance Characteristics Section are suitable for assay verification.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Interpretation of Staining:

Positive Tissue Control:

The positive tissue control stained with indicated antibody should be examined first to ascertain that all reagents are functioning properly. The appropriate staining of target cells (as indicated above) is indicative of positive reactivity. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

The color of the reaction product may vary depending on substrate chromogens used. Refer to substrate package inserts for expected color reactions. Further, metachromasia may be observed in variations of the method of staining.¹¹

When a counterstain is used, depending on the incubation length and potency of the counterstain used, counterstaining will result in a coloration of the cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results. Refer to protocol(s) for recommended counterstain.

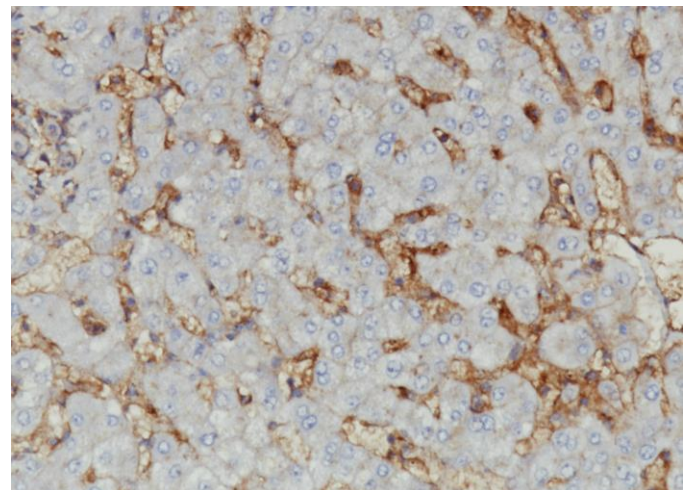
Negative Tissue Control:

The negative tissue control should be examined after the positive tissue control to verify the specificity of the 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/cellular components. If specific staining (false positive staining) occurs in the negative external tissue control, results with the patient specimen should be considered invalid.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue:

Examine patient specimens stained with indicated antibody last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

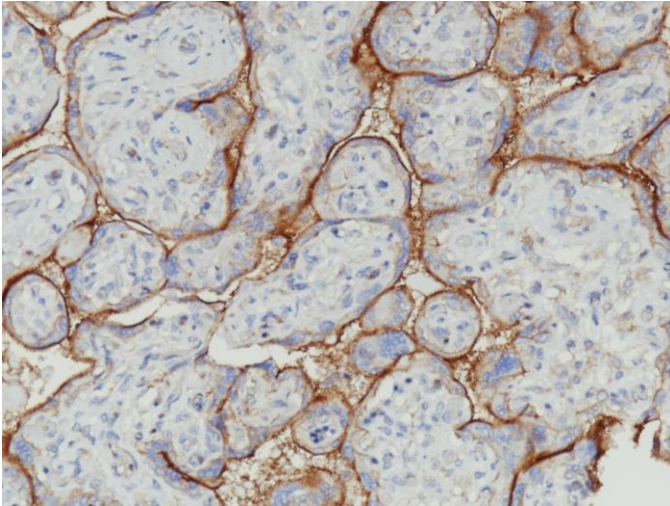


Liver cancer stained with CD47 [EPR21794] antibody

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Placenta stained with CD47 [EPR21794] antibody

Refer to Summary and Explanation, Limitations, and Performance Characteristics for specific information regarding indicated antibody immunoreactivity.

Limitations:

General Limitations:

1. For *in vitro* diagnostic Use
2. This product is for professional use only: Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
3. 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 be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.¹²
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology, and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents, and methods to interpret all the steps used to prepare and interpret the final IHC preparation.
6. The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.
7. This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.

8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.¹³
9. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues.¹⁴ Contact Biocare's Technical Support at 1-800-542-2002, or via the technical support information provided on biocare.net, with documented unexpected reaction(s).
10. Normal/nonimmune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
11. False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudo peroxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g., liver, breast, brain, kidney) depending on the type of immunostain used.¹²

Product Specific Limitations:

No additional limitations to the product have been identified.

Performance Characteristics:

Sensitivity, specificity, and cross-reactivity are summarized in Tables 1 and 2, respectively.

Reproducibility:

A reproducibility study was conducted with four samples over five runs on five separate days by at least two operators. Staining was consistent across all staining on the samples.

Inter-run reproducibility of staining was determined by staining slides containing the same normal tissue on 4 number of runs. Staining was equal across all runs.

Immunoreactivity:

The following positive and negative immunoreactivities have been demonstrated in Tables 1 and 2 below.

The list provided below is not exhaustive but characterizes the types of immunoreactivities observed with the indicated antibody.

Summary of Expected Results:

Normal adult human tissue shows very broad expression with staining only absent in the heart, lung, liver, mesothelial cells, and pericardial cells (see table 2). Table 1 highlights a moderate to high frequency in some forms of renal cancer, with high frequency in liver cancer, invasive urothelial carcinoma. Additionally, peritoneal and omental carcinomas show a high prevalence of CD47 staining, as do endometrial cancers.

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Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases	Comments
Renal Clear Cell Carcinoma	8	33	Low intensity
Invasive urothelial carcinoma	11	11	High intensity
Chromophobe carcinoma	3	5	Variable intensity
Papillary Renal Cell CA	3	9	Variable intensity
Other Renal cancer	12	14	Variable intensity
Peritoneum and omentum tumor	38	40	High intensity
Endometrial Carcinoma	11	12	High intensity and frequency
Liver Carcinoma	24	35	Variable intensity

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases	Notes
Cerebrum	6	6	Moderate intensity
Cerebellum	3	3	High intensity
Adrenal	3	3	Low intensity and frequency
Ovary	3	3	Low frequency in stroma
Pancreas	2	3	In ducts and islets
Lymph Node	3	3	Moderate intensity
Trachea	2	3	Submucosal
Testis	3	3	Moderate expression
Thyroid	3	3	Very low intensity but widely expressed
Breast	3	3	Low intensity
Spleen	3	3	High intensity
Tonsil	3	3	High intensity
Thymus	3	3	Very low intensity but widely expressed
Bone Marrow	1	3	Low intensity
Lung	0	3	N/A
Heart	0	3	N/A
Esophagus	2	3	Low intensity in basal cells
Stomach	3	3	Glandular cells
Small Intestine	0	0	N/A
Colon	3	3	Low intensity in epithelia, high in nerves
Liver	0	11	N/A
Salivary Gland	2	3	Ductal staining
Kidney	5	6	Glomerular staining
Prostate	2	3	High intensity staining
Uterus	2	3	Epithelial cell staining
Cervix	1	3	N/A
Skeletal Muscle	0	3	N/A
Skin	0	3	N/A
Peripheral Nerve	2	3	N/A
Lining Cells	0	3	N/A
Eye	1	3	N/A
Larynx	1	2	Basal Cell and submucosal cell staining

Troubleshooting:

1. No staining of any slides – Check to determine appropriate positive control tissue, antibody, and detection products have been used.
2. Weak staining of all slides – Check to determine appropriate positive control tissue, antibody, and detection products have been used.
3. Excessive background of all slides – There may be high levels of endogenous biotin (if using biotin-based detection products), endogenous HRP activity converting chromogen to colored end product

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(use peroxidase block), or excess non-specific protein interaction (use a protein block, such as serum- or casein-based blocking solution).

4. Tissue sections wash off slides during incubation – Check slides to ensure they are positively charged.
5. Specific staining too dark – Check protocol to determine if proper antibody titer was applied to slide, as well as proper incubation times for all reagents. Additionally, ensure the protocol has enough washing steps to remove excess reagents after incubation steps are completed.

References:

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