Antigen Retrieval 1 (AR1), high pH

Antigen Retrieval
901-OPRI6006-090622

BIOCARE MEDICAL

Available Product Formats	
Catalog Number	Volume
OPRI6006T60	60 tests

Intended Use:
For *in vitro* Diagnostic Use

The Antigen Retrieval 1 (AR1), high pH is intended for laboratory professional use in heat-induced antigen retrieval (HIER) of formalin-fixed paraffin-embedded (FFPE) tissues for use in either manual or automated immunohistochemistry (IHC) staining protocols. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
The Antigen Retrieval 1 (AR1), high pH is designed as a Tris-EDTA-based buffer used in heat-induced antigen retrieval (HIER) of formalin-fixed, paraffin-embedded (FFPE) tissues. HIER is the process of heating the slide-mounted specimen material in an antigen retrieval solution, followed by a cooling-off period. Retrieval solutions with paraffin emulsifiers (surfactants) followed by a rinsing reagent can deparaffinize and retrieve tissues, thus eliminating the deparaffinization step with xylenes and alcohols.

Most immunohistochemistry (IHC) specimens are routinely fixed in formalin and, while formalin provides excellent preservation of morphology, it can greatly diminish the sensitivity to IHC. Formalin creates cross-links that alter protein biochemistry and can block antigenic sites. Antigen retrieval is needed to break these cross-links and expose the antigen's epitope. Biocare's Antigen retrieval reagents are used in heat-induced antigen retrieval (HIER) of formalin-fixed, paraffin-embedded (FFPE) tissues. The antigen retrieval process has many variables, including the target antigen, the antibody, fixation time, and tissue processing as well as the antigen retrieval method. For the HIER method, which is the process of heating the slide-mounted specimen material in an antigen retrieval solution, followed by a cooling-off period, the pH and composition of the retrieval solution are important factors.

Antigen Retrieval 1 (AR1), high pH is capable of partially breaking the covalent bonds of formalin-fixed tissue, as part of an IHC staining procedure on the ONCORE Pro Automated Slide Stainer. Pretreatment of FFPE tissues with Antigen Retrieval 1 (AR1), high pH can significantly increase staining intensity. The solution is provided ready-to-use and is intended to be applied as defined by the staining protocols on the ONCORE Pro Automated Slide Stainer.

Principle of Procedure:
This reagent when used as heat-induced epitope retrieval solution applied to pretreated formalin-fixed, paraffin-embedded tissue sections enhances primary antibody staining.

Materials and Methods:

Reagents Provided:

Kit Catalog No.	Component Description	Quantity x Volume
OPRI6006T60	Antigen Retrieval 1 (AR1), high pH (OPRI6006T30)	2 x 14.5 mL

Reconstitution, Mixing, Dilution, Titration:
The Antigen Retrieval 1 (AR1), high pH is optimized for use with Biocare antibodies and ancillary reagents. No reconstitution, dilution, or mixing is required.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffered Tris-EDTA solution, pH 9.0. 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
Peroxidase block (optional)
Protein block (optional)
Detection probe and polymer
Negative control reagents
Chromogens
Hematoxylin (counterstain)
Bluing reagent
Mounting medium
Coverglass
Light Microscope (40-400X magnification)
ONCORE Pro Automated Slide Stainer

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

Storage and Stability:
Store at 2ºC to 8ºC. 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. The reagent(s) are ready-to-use and should not be diluted. 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 antigen retrieval reagent is suspected, contact Biocare's Technical Support at 1-800-542-2002 or via the technical support information provided on biocare.net.

Specimen Preparation prior to antigen retrieval:
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]

Warning and Precautions:
1. Handle materials of human or animal origin as potentially biohazardous and dispose such materials with proper precautions. In the event of

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exposure, follow the health directives of the responsible authorities where used.[6,7]
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.[8]
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. The antigen retrieval reagents are optimized and ready to use.
7. Follow local and/or state authority requirements for method of disposal.
8. The SDS is available upon request and is located at http://biocare.net.
9. Report any serious incidents related to this device by contacting the local Biocare representative and the applicable competent authority of the Member State or country where the user is located.

Instructions for Use:
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]

The Antigen Retrieval 1 (AR1), high pH reagent is optimized for use with Biocare antibodies and ancillary reagents. Refer to the primary antibody information for use for recommended protocols and conditions for use. Incubation times and temperatures will vary depending on the specific antibody protocol followed.

Antigen Retrieval 1 (AR1), high pH is provided in vials ready for use on the ONCORE Pro Automated Slide Stainer. Uncap the vial and place in the ONCORE Pro reagent tray. The ONCORE Pro will apply reagent as required in the selected protocol. Refer to the appropriate antibody data sheet for the recommended staining protocol. Refer to the ONCORE Pro Automated Slide Staining System User Manual for detailed instructions on instrument operation and additional protocol options.

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). 2011[9]

Positive Tissue Control:
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 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 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 an antibody produced and prepared (i.e. diluted to same concentration using same diluent) for use 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. 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 Program[10] for Immunohistochemistry and/or the NCCLS IHC guideline[11]. 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:
A primary antibody works in conjunction with ancillary reagents to produce a colored reaction at the antigen sites localized by the primary antibody. Diluent ancillary reagents assist with providing a pH buffered environment to facilitate primary antibody binding in the antibody-antigen specific staining reaction. Prior to interpretation of patient results, the staining of controls must be evaluated by a qualified pathologist. Negative controls are evaluated and compared to stained slides to ensure any staining observed is not a result of nonspecific interactions.

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.[10]
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.

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.[11]
4. 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.
5. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[12]
6. 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.[13] 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).

Product Specific Limitations:
No additional product specific limitations identified.

Performance Characteristics:
Staining was performed using protocols provided in the antibody specific instructions for use or as specified. Sensitivity and specificity of staining was evaluated across a range of normal and neoplastic tissue types evaluated during development of primary antibodies.

Reproducibility:
The reproducibility of Biocare's antigen retrieval reagents is verified through a measurement of intermediate precision in which various reagent lots were tested over an extended period of time using various operators, analysts, reagent lots, tissue samples, and equipment. The staining obtained for each diluent reagent evaluated was consistent and performed as expected.

Troubleshooting:
1. No staining of any slides – Check to determine appropriate positive control tissue, antibody, and detection products have been used. Check for incomplete or improper wax removal or pretreatment.
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 (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.

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