

Folate Receptor alpha IHC Assay Kit

Prediluted Monoclonal Antibody with HRP Detection Kit

Control Number: 902-BRR4006K-073117

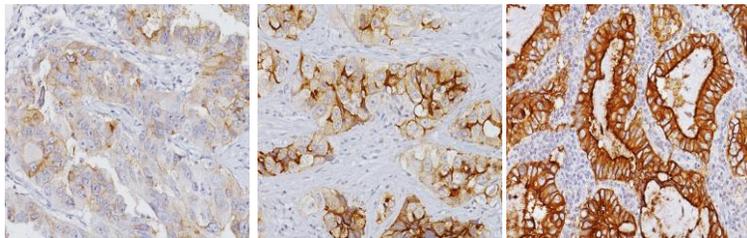
Catalog Number: BRR 4006K AA
Description: approximately 32 tests

Intended Use:
 For Research Use Only. Not for use in diagnostic procedures.

Summary & Explanation:

Mouse anti-human Folate Receptor alpha monoclonal antibody [26B3.F2] specifically recognizes the alpha isoform of Folate Receptor. Folate Receptor alpha is primarily expressed in the apical surface of some polarized epithelial cells of normal tissues (such as kidney, lung, breast, and salivary glands) and on many cancer cells of epithelial origin; namely, ovarian cancer, thyroid cancer, non-small cell lung adenocarcinoma (NSCLC) and endometrial cancer.

In the most widely studied tumor, epithelial ovarian cancer, expression of FRalpha increases with tumor stage [14], and is associated with decreased survival [13]. However, in NSCLC, FRalpha has been shown to be specific for adenocarcinomas relative to squamous cell carcinoma and other histologic subtypes, and increased expression has been correlated to increased survival [1,5,6,11].



Lung adenocarcinoma, 1+ staining Lung adenocarcinoma, 2+ staining Lung adenocarcinoma, 3+ staining

Principle of Procedure:

The Folate Receptor alpha IHC Assay Kit contains reagents required to complete an IHC staining procedure for formalin-fixed, paraffin-embedded specimens. Following incubation with the primary monoclonal antibody to human folate receptor alpha protein, or the negative control reagent, the validated protocol employs two-step, ready-to-use, visualization reagents. A rabbit anti-mouse secondary antibody is used to detect the primary antibody, and a horseradish peroxidase (HRP) labeled goat anti-rabbit micro-polymer is used to recognize the rabbit immunoglobulins present in the secondary antibody. The subsequently added DAB chromogen is converted by the HRP enzyme of the micro-polymer into a visible reaction product (brown precipitate) at the antigen site. The specimen may then be counterstained, dehydrated, cleared and coverslipped. Results are interpreted by using a light microscope.

Folate Receptor alpha IHC Assay Kit (BRR4006K) is applicable for use in a manual staining procedure, or on an automated stainer (e.g. Dako Autostainer). The number of tests (approximately 30) is based on the use of 200 µl (4-5 drops) of each reagent per slide (except Diva Decloaker).

Materials Provided:

1. **Peroxidized 1.** BRR968G, 2 x 6 mL
Buffered solution containing 3% hydrogen peroxide, plus stabilizer and proprietary components; ready-to-use.
2. **Background Punisher,** BRR974G, 2 x 6 mL
Proprietary combination of proteins in modified PBS with preservative and surfactant; ready-to-use.

Materials Provided cont'd:

3. **Folate Receptor alpha.** APR3005AA, 1 x 6 mL
Purified mouse anti-human monoclonal antibody in Tris buffer pH 6.1 to 6.3, with bovine serum albumin as a protein carrier and 0.09% sodium azide as a preservative; color-coded green; ready-to-use. (Licensed from Morphotek Inc. Patent pending.)
 - **Clone:** 26B3.F2
 - **Isotype:** IgG1/ kappa
 - **Species Reactivity:** Human, others not tested
 - **Antibody Category:** Adenocarcinoma (breast, lung, endometrial, ovarian)
 - **Epitope/ Antigen:** Folate Receptor alpha protein
 - **Cellular Localization:** Membrane, cytoplasmic
 - **Total protein concentration:** Call for lot specific IgG concentration.
4. **FRalpha Negative Control Reagent.** BRR4008AA, 1 x 6 mL
Purified mouse IgG1/kappa [MOPC-21] in Tris buffer pH 6.1 to 6.3, with bovine serum albumin as a protein carrier and 0.09% sodium azide as a preservative; color-coded red; ready-to-use.
5. **MACH 4 Mouse Probe.** BRR534DG, 2 x 6 mL
Rabbit anti-mouse secondary antibody in a buffer with protein carrier and preservative, color-coded yellow; ready-to-use.
6. **MACH 4 HRP Polymer.** BRR534BG, 2 x 6 mL
Horseradish peroxidase (HRP) labeled goat anti-rabbit micro-polymer in a buffer with protein carrier and preservative; color-coded orange; ready-to-use.
7. **Betazoid DAB Substrate Buffer.** BRR900BH, 1 x 25 mL
Clear, colorless buffer with 0.02% hydrogen peroxide.
8. **Betazoid DAB Chromogen.** BRR900AC, 1 x 1 mL
3,3'-diaminobenzidine tetrahydrochloride chromogen supplied as golden brown to purple viscous liquid solution.
9. **Mixing Vial.** RMVL103, 1 each
10. **Diva Decloaker, 10X.*** BRR2004C, 1 x 100 mL
10X concentrated heat retrieval solution incorporating a high temperature pH indicator; color-coded orange. Ready-to-use Diva Decloaker solution is pH 6.2.
*Diva Decloaker should be stored at room temperature. Additional Diva Decloaker may be ordered separately.

Materials Required But Not Supplied:

- TBS Wash Buffer (BRR945, BRR946)
- Hematoxylin
- Microscope slides, positively charged
- Positive and negative tissue controls
- Desert Chamber (Drying oven)
- Xylene or xylene substitute, such as Slide Brite
- Ethanol or reagent alcohol
- Decloaking Chamber (pressure cooker) or water bath
- Deionized or distilled water
- Bluing reagent
- Mounting medium
- Light microscope

Storage and Stability:

- Store Folate Receptor alpha IHC Assay Kit at 2-8°C.
- Store Diva Decloaker 10X at room temperature (20-25°C).
- Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Do not expose DAB Chromogen to strong light or direct sunlight.

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Specimen Preparation:

The Folate Receptor alpha IHC Assay Kit is suitable for use with formalin-fixed, paraffin-embedded (FFPE) tissues. Standard methods of tissue processing and fixation should be used for all specimens. The recommended fixative is 10% neutral-buffered formalin.

Staining Procedure:

All reagents should be equilibrated to room temperature (20-25°C) prior to staining. Do not allow tissue sections to dry during the staining procedure. Dried tissue sections may display increased nonspecific staining.

Deparaffinization and Rehydration:

Should be performed at room temperature (20-25°C).

1. Immerse slides in 3 changes of xylene or xylene substitute for 3 minutes each.
2. Tap off excess liquid and immerse slides in 3 changes of reagent alcohol for 3 minutes each.
3. Tap off excess liquid and immerse slides in 95% alcohol for 1 minute.
4. Tap off excess liquid and immerse slides in 80% alcohol for 1 minute.
5. Tap off excess liquid and rinse slides in reagent-quality water for a minimum of 1 minute, change water and repeat once. Proceed with the antigen retrieval procedure.

Peroxidase Block:

Incubate tissue sections for 5 minutes at RT with Peroxidized 1 (BRR968). Rinse in reagent-quality water.

Heat Pretreatment (Antigen Retrieval) Protocol:

Preferred method: 40 minutes at 95°C (preheated), with a cool down time of 20 minutes.

1. Add a consistent volume of reagent-quality water to pressure cooker pan (500 mL if using Biocare's Decloaking Chamber).
2. Prepare a sufficient quantity of Diva Decloaker solution by diluting it 1:10 (1 part Diva to 9 parts of reagent-quality water) for the staining procedure planned.
3. Fill slide containers with the prepared Diva Decloaker.
4. Place the Diva Decloaker retrieval solution (without slides) into the Decloaking Chamber; preheat to 95°C for 30 minutes.
 - Alternatively, if using Biocare's NxGen Decloaking Chamber, use Program 4 for 95°C for 40 minutes
 - Keep the slides in reagent quality water until the retrieval solution is ready.
5. Place slides into the preheated solution and retrieve at 95°C for 40 minutes.
6. Allow slides to cool for 20 minutes in the Decloaking Chamber, with the lid removed, then wash in reagent quality water.
 - Check Diva Decloaker solution for color change. The solution should be orange in color, which indicates the correct pH at high temperature. Should the pH rise above 7.0, the solution will turn pink to red.
7. Immerse slides in wash buffer for 3 (±1) minutes after antigen retrieval prior to staining.

Notes:

- Diva Decloaker solution may be used only once. Discard after use.
- Use blank slides to fill staining jars or other appropriate slide holders.
- Alternatively, a water bath may be used for antigen retrieval procedure at 95±2°C for 40 minutes, with a 20 minute cool down time.

Protein Block:

Incubate tissue sections for 5-10 minutes at RT with Background Punisher (BRR974). Wash in TBS buffer.

Primary antibody:

Incubate tissue sections for 30 minutes at RT with Folate Receptor alpha antibody (APR3005AA) or FRalpha Negative Control Reagent (BRR4008AA). Wash in TBS buffer.

Probe:

Incubate tissue sections for 10 minutes at RT with MACH 4 Mouse Probe (BRR534D). Wash in TBS buffer.

Polymer:

Incubate tissue sections for 10 minutes at RT with MACH 4 HRP Polymer (BRR534B). Wash in TBS buffer.

Chromogen:

1. Prepare DAB working solution by mixing 1 drop (~32 µl) of Betazoid DAB Chromogen into 1.0 mL of Betazoid DAB Substrate Buffer.
2. Apply DAB working solution to tissue sections. Incubate for 5 minutes at RT. Rinse in distilled water.

Counterstain:

Counterstain with hematoxylin. Rinse with distilled water. Apply Bluing Reagent. Rinse with distilled water.

*Strong counterstaining may mask FRalpha staining.

Mounting:

Use a permanent mounting medium for coverslipping.

Dako Autostainer Protocol:

Following antigen retrieval, staining may be performed on the Dako Autostainer. Refer to the Dako Autostainer User Manual for further information.

1. Create a protocol using the steps and incubation times indicated above. (Figure 1).
2. Place the reagent vials in the Dako Autostainer reagent rack according to the computer generated reagent map.
3. Load the slides onto the Dako Autostainer according to the computer generated slide map.
4. Begin the run.
5. At the end of the run, remove slides from the Dako Autostainer.
6. Rinse slides in reagent-quality water.

Figure 1.



Slides	File	Edit lists	Copy	Auto						
Slide #	Dispense	End. Enz. Block	Pretreatment	Protein Block	Primary Antibody	Secondary Reagent	Tertiary Reagent	Substrate	Wash	Wash
1	100 µl	100 µl	Diva 95/40 (Manual Step)	B. Punisher 10'	FRalpha 30'	M4 HRP pro 10'	M4 HRP pol 10'	Bet DAB 5'	100 µl	100 µl
2	100 µl	100 µl	Diva 95/40 (Manual Step)	B. Punisher 10'	FR Neg 30'	M4 HRP pro 10'	M4 HRP pol 10'	Bet DAB 5'	100 µl	100 µl

Quality Control:

Differences in tissue fixation, processing, and embedding in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls.

Positive Tissue Control:

The recommended positive tissue control for use with Folate Receptor alpha is ovarian serous papillary adenocarcinoma or lung adenocarcinoma. Controls should be fresh biopsy/surgical specimens, fixed, processed, and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are used to indicate correctly prepared tissues and proper staining techniques. One positive tissue control should be included for each set of test conditions in each staining run. A tissue with weak positive staining is more suitable for optimal quality control and to detect minor levels of reagent degradation.

Ultimately, freshly cut control slides should be used for each staining run. Prepared weakly positive tissue control slides should not be stored for more than 7 days. Strong positive controls may be stored for up to 30 days. Recommended storage conditions for tissue control slides: 2-8°C.

If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control:

The recommended negative control tissue for use with Folate Receptor alpha is normal liver. Negative tissue controls (fixed, processed, and embedded in the same manner as the patient sample(s)) should be used with each staining run to verify the specificity of the primary antibody.

Alternatively, the variety of different cell types present in many tissue sections frequently offers negative control sites, but this should be verified by the user. If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid, and the test should be repeated.

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Negative Control Reagent:

Use the supplied FRalpha Negative Control Reagent (BRR4008) 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. The incubation period for the negative control reagent should correspond to that of the primary antibody.

Assay Verification:

Prior to initial use of Folate Receptor alpha IHC Assay in a diagnostic procedure, the user should verify the kit specificity by testing it on a series of in-house tissues with known IHC 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 requirements of the CAP Certification Program for Immunohistochemistry [24] and/or NCCLS IHC Guideline [26]. 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.

Staining Interpretation:

Slide evaluation should be performed by a pathologist using a light microscope. For determination of FRalpha protein expression in tumor tissues, only membrane staining patterns should be evaluated. Scoring is based on examining all tumor cells on the slide.

Performance Characteristics:

Normal tissues:

[26B3.F2] stains the cytoplasm and membrane of epithelial cells of various normal tissues such as pancreas, lung, salivary gland, kidney, pituitary gland, and breast.

Tumor tissues:

[26B3.F2] staining is restricted to several tumor types of epithelial origin, namely, ovarian cancer, thyroid cancer, non-small cell lung adenocarcinoma and endometrial cancer.

Reproducibility:

Reproducibility testing on lung adenocarcinoma tissues, including intra-run (triplicates within a single staining run), inter-run (the same tissues on 5 different days) and inter-site (3 different sites), was performed using the FRalpha IHC Assay Kit on Dako autostainer. Test slides from the same specimen displayed comparable staining intensity in each run.

Precautions:

1. For use by professional laboratory personnel that have been properly trained in its use.
2. Do not substitute reagents from other manufacturers.
3. This product contains less than 0.1% sodium azide. Sodium azide (NaN_3) 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 [25].
4. This product contains less than 0.25% Proclin 950 and Proclin 300. Overexposure to Proclin can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract.
5. This product contains 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen. DAB is known to be a suspected carcinogen. DAB may cause sensitization of skin. Wear appropriate personal protective equipment and clothing.
6. 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.
7. Avoid contact with skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
8. The SDS is available upon request, and is located at <http://biocare.net>.

General Limitations:

1. 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.
2. Excessive or incomplete counterstaining may compromise proper interpretation of results.

General Limitations cont'd:

3. This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.
4. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
5. 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.
6. 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.
7. The reagents and instructions supplied in this system have been designed for optimal performance. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

Troubleshooting Guide:

No Staining

1. Critical reagent (such as primary antibody) omitted.
2. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, in the wrong order, or the wrong dilution.
4. Insufficient amount of antigen in tissue.
5. Reagent incubation times too short.
6. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under-fixed.
2. Reagents incubation times too short.
3. Low expression of antigen in tissue.
4. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong dilution.
5. Excessive rinsing during wash steps.
6. Incorrect procedure in reagent preparation.
7. Improper procedure in test steps.

Non-specific or High Background Staining

1. Tissue is either over-fixed or under-fixed.
2. Tissue was inadequately rinsed.
3. Deparaffinization incomplete.
4. Tissue damaged or necrotic.
5. Tissues were allowed to dry.

Tissues Falling Off

1. Slides were not positively charged.
2. A slide adhesive was used in the water bath.
3. Tissue was not dried properly.
4. Tissue contained too much fat.

Specific Staining Too Dark

1. Reagent incubation times are too long.



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References:

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