



Folate Receptor alpha IHC Assay Kit

Prediluted Monoclonal Antibody with HRP Detection Kit

Control Number: 901-IP14006K-081717

Catalog Number:

IPI4006K G10

Description:

approximately 32 tests on intelliPATH™
Automated Slide Stainer.

Intended Use:

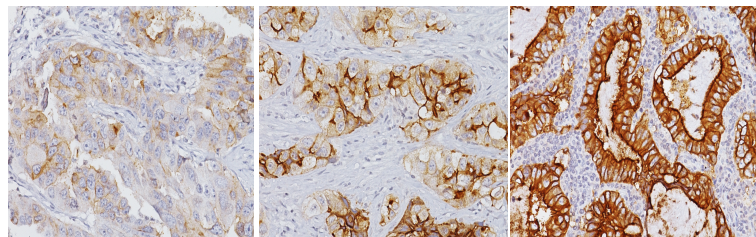
For In Vitro Diagnostic Use.

The Folate Receptor alpha IHC Assay Kit is intended for use in immunohistochemical procedures for the identification of folate receptor alpha (FRalpha) expression in formalin-fixed, paraffin-embedded (FFPE) tissue sections, using an intelliPATH™ Automated Slide Stainer. 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.

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.

Protocol Statement:

Folate Receptor alpha IHC Assay Kit (IPI4006K) has been formulated for use with Biocare's intelliPATH™ Automated Slide Stainer. Reagent volumes have been adjusted specifically for use on the intelliPATH™ Automated Slide Stainer. The number of tests (approximately 32) is based on the use of 300 µL of each reagent per slide (excluding Diva Decloaker). Please refer to the intelliPATH™ User Manual for further information to navigate intelliPATH™ software and stainer.

Materials provided:

- intelliPATH™ Peroxidase Blocking Reagent.** IPB5000G20, 1 x 20 mL
Buffered solution containing 1% hydrogen peroxide, plus stabilizer and proprietary components; ready-to-use.
- intelliPATH™ Background Punisher.** IP974G20, 1 x 20 mL
Proprietary combination of proteins in modified PBS with preservative and surfactant; ready-to-use.
- Folate Receptor alpha.** IPI3005G10, 1 x 10 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.
- FRalpha Negative Control Reagent.** IPI4008G10, 1 x 10 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.
- intelliPATH™ Mouse Secondary Reagent.** IPSC5001G20, 1 x 20 mL
Rabbit anti-mouse secondary antibody in a buffer with protein carrier and preservative, color-coded yellow; ready-to-use.
- intelliPATH™ Universal HRP Tertiary Reagent.** IPT5002G20, 1x 20 mL
Horseradish peroxidase (HRP) labeled goat anti-rabbit micro-polymer in a buffer with protein carrier and preservative; color-coded orange; ready-to-use.
- intelliPATH™ DAB Buffer.** IPBF5009G20, 2 x 20 mL
Clear, colorless buffer with 0.02% of hydrogen peroxide.
- intelliPATH™ DAB Chromogen.** IPC5008G3, 1 x 3 mL
3,3'-diaminobenzidine tetrahydrochloride chromogen supplied as golden brown to purple viscous liquid solution.
- intelliPATH™ Mixing Vial.** IP5028MV, 1 each
- Diva Decloaker, 10X.*** DV2004LX, 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 Automation Wash Buffer, 20X TWB945
Hematoxylin
Microscope slides, positively charged
Positive and negative tissue controls
Desert Chamber (Drying oven)
Xylene or xylene substitute, such as Slide Brite SBT
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.

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 (e.g. Coplin jars) 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.
 - 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, 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.

intelliPATH Automated Slide Stainer Protocol:

Following antigen retrieval, staining is performed on the intelliPATH™ Automated Slide Stainer. Refer to intelliPATH™ Automated Slide Stainer User Manual for further information.

1. Add a New Case.
2. Select Folate Receptor alpha protocol from All Protocols, check positive and negative controls if needed.
3. Load the slides on intelliPATH™ Automated Slide Stainer.
4. Load the reagent vials and mixing vials on intelliPATH™ Automated Slide Stainer.
5. Fill Buffer, DI water carboys and empty waste carboys, if needed.
6. Begin the run.

Figure 1.

Protocol Properties

Protocol Name

FRalpha

Template

Mouse HRP

Type

User

Dispense Zone

Total Volume/Slide

300

▼

Apply Volume

Details :

#	Name	Blow	Reagent	Volume	Time	Wash	Count
1	Block	✓	IP Peroxidase (...)	300	00:05	Buffer1	1
2	Block	✓	IP Background ...	300	00:10	Buffer1	1
3	Pre-Treatment	✓	Diva (DV2004)	300	00:00	Buffer1	1
4	Antibody	✓	Folate Recepto...	300	00:30	Buffer1	1
5	Secondary	✓	Mouse Seconda...	300	00:10	Buffer1	1
6	Tertiary	✓	Univ HRP Tertia...	300	00:10	Buffer1	2
7	Chromogen	✓	IP FLX DAB (IPK...	300	00:05	DI Water	2
8	Counter Stain	✓	IP FLX Hematox...	300	00:05	Buffer1	2
9	Wash				00:00	Buffer1	1
10	Wash				00:00	DI Water	1

Counterstain:

Counterstaining with hematoxylin can be performed on the intelliPATH™ or manually after the slides are removed from the autostainer. Strong counterstaining may mask FRalpha staining.

Mounting:

Use a permanent mounting medium for coverslipping.

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. 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.

Negative Control Reagent:

Use the supplied FRalpha Negative Control Reagent (IP14008G10) 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



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Assay Verification cont'd:

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 FRalpha IHC Assay Kit on intelliPATH Automated Stainer. Test slides from the same specimen displayed comparable staining intensity in each run.

Precautions:

1. For professional users only.
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 and Warranty:

1. IHC is a multistep diagnostic process that requires specialized training in the election of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
2. 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.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining or its absence should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative staining must be complemented by morphological studies and proper controls as well as other diagnostic tests. 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.
5. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.

General Limitations and Warranty cont'd:

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.
7. 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.
8. 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.
9. There are no warranties, expressed or implied, which extend beyond this description. Biocare as a manufacturer of this product is not liable for property damage, personal injury, or economic loss caused by this product.

Restrictions or Training Requirement:

Folate Receptor alpha IHC assay is optimized for use with Biocare Medical's automated staining system - the intelliPATH™. Prior to undertaking this methodology, users must be trained in immunohistochemical techniques on the intelliPATH™.

Troubleshooting:

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

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 during loading on intelliPATH™.

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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