



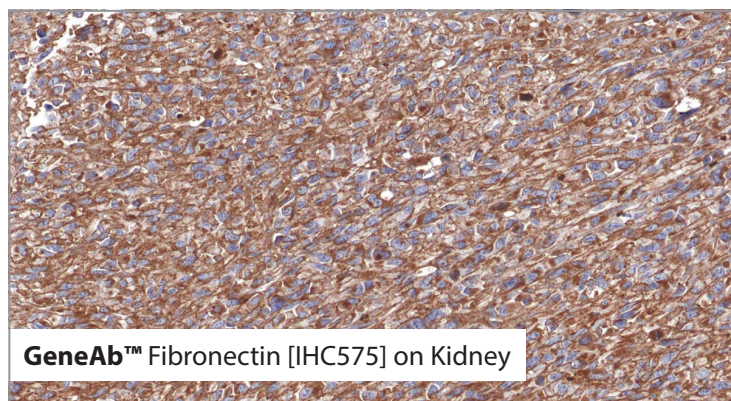
GeneAb™

# Fibronectin

**Clone:** IHC575

**Source:** Mouse Monoclonal

**Positive Control:** Kidney



## Product Information

REF	Description
IHC575-100	0.1 ml, Concentrate
IHC575-1	1 ml, Concentrate
IHC575-7	7 ml, Predilute
IHC575-PC	3 Positive Control Slides

## 1. Intended Use

This antibody is intended for *in vitro* diagnostic (IVD) use.

The Fibronectin [IHC575] antibody is intended for qualified laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods. Use of this antibody is indicated, subsequent to clinical differential diagnoses of diseases, as an aid in the identification of hepatocellular carcinoma within the context of antibody panels, the patient's clinical history and other diagnostic tests evaluated by a qualified pathologist.

## 2. Summary and Explanation

Fibronectin is a glycoprotein that contributes to cell adhesion, migration, and metastasis. Renal cancer cells exhibit higher expression of fibronectin, therefore Anti-Fibronectin is useful for assessing the progression and aggressiveness of renal cancer cells.

## 3. Principles and Procedures

Visualization of the antigen present in tissue sections is accomplished in a multi-step immunohistochemical staining process, in conjunction with a horseradish peroxidase (HRP) or alkaline phosphatase (AP) linked detection system. The process involves the addition of the stated antibody (primary antibody) to a tissue slide, followed by a secondary antibody (linked to an enzyme complex) which specifically binds to the primary antibody. A chromogenic substrate is then added which reacts with the enzyme complex, resulting in a colorimetric reaction at the site of the antigen. Results are interpreted using a light microscope.

## 4. Materials and Methods

Product Format	Optimized Buffer Composition
Predilute	Antibody Diluent Buffer
Concentrate	Tris Buffer, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide

*Note:* The recommended working dilution range is 1:50 - 1:200.

### Reconstitution, Mixing, Dilution, and Titration

The prediluted antibody does not require any mixing, dilution, reconstitution, or titration; the antibody is ready-to-use and optimized for staining.

The concentrated antibody requires dilution in the optimized buffer, to the recommended working dilution range (see table above).

## Storage and Handling

Store at 2-8°C. Do not freeze.

When stored correctly, the antibody is stable until the date indicated on the label.

To ensure proper stability and delivery of the antibody after each run, replace the cap and immediately place the bottle in a refrigerator in an upright position.

Positive and negative controls should be simultaneously run with unknown specimens, as there are no conclusive characteristics to suggest instability of the antibody. If such an indication of instability is suspected, contact GenomeMe® Customer Service at [info@GenomeMe.ca](mailto:info@GenomeMe.ca).

## Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4µm), and placed on a glass slide that is positively charged. The prepared slide may then be baked for a minimum of 30 minutes in a 53-65°C oven (do not exceed 24 hours).

*Note:* Performance evaluation has been shown on human tissues only. Variable results may occur due to extended fixation time or special processes of specific tissue preparations.

## 5. Instructions For Use

### Recommended Staining Protocols for the Fibronectin [IHC575] antibody:

#### **Manual Use:**

1. **Pretreatment:** Perform heat-induced epitope retrieval (HIER) at pH 9 for 10 to 30 minutes.
2. **Peroxide Block:** Block in peroxidase blocking solution for 5 minutes at room temperature. (Not required if using Alkaline Phosphatase System.)
3. **Primary Antibody:** Apply antibody directly (Predilute) or dilute antibody at 1:50-1:200 (Concentrate) before applying. Incubate antibody for 10 to 30 minutes at room temperature.
4. **Secondary Antibody:** Incubate for 20 to 30 minutes at room temperature.
5. **Substrate Development:** Incubate DAB or Fast Red for 5 to 10 minutes at room temperature.
6. **Counterstain:** Counterstain with hematoxylin for 0.5 to 5 minutes, depending on the hematoxylin used. Rinse with distilled water and blueing solution for 30 seconds.
7. Dehydrate and apply coverslip.

#### **Automated Staining System:**

The stated primary antibody has been optimized and validated using the BOND-MAX fully automated IHC & ISH stainer manufactured by Leica Biosystems, applying IHC Protocol F. The following edits are recommended for the protocol:

- a) Marker Incubation Time: 30 minutes
- b) Heat-induced epitope retrieval (HIER) is recommended using Leica Bond ER Solution 2 for 30 minutes.
- c) Move Peroxide Block step to after Polymer and before Mixed DAB Refine.

For all other automated IHC staining systems, refer to the corresponding user manual for specific instructions.

## 6. Quality Control Procedures and Interpretation of Results

The immunohistochemical staining process results in a colorimetric reaction at the site of the antigen, localized by the primary antibody. A qualified pathologist must interpret the patient results only once the positive and negative control tissues have been analyzed.

### Positive Control Tissue

A positive control tissue must be run with each staining procedure, and must be prepared and fixed identically to the test sections in order to provide control for all test variables, including fixation and tissue processing. The positive control tissue should be fresh autopsy, biopsy, or surgical specimens. For optimal quality control and to allow detection of lesser levels of reagent degradation, a tissue with weaker positive staining is advisable. Kidney tissue can be used as positive control tissue for the Fibronectin [IHC575] antibody. Where applicable, tissue that contains cells or tissue components that stain both positively and negatively may serve as both the positive and negative control tissue.

Once stained, the positive control tissue should be analyzed to ensure appropriate positive staining is observed and all reagents are functioning properly. Positive reactivity requires the observation of an

appropriate colorimetric reaction at the site of the antigen within the target cells. Counterstaining will result in a blue coloration, which may be pale to dark depending on the length of the incubation time and potency of the hematoxylin.

If positive staining as defined herein is not observed, the results obtained with the patient or tissue specimen must be treated as invalid. The positive control tissue should not be used as an aid in the diagnoses of patient samples, but rather solely as a measure of performance of the reagents and validity of obtained results.

### Negative Control Tissue

The same tissue used for the positive control tissue may be used as the negative control tissue.

Most tissue sections offer internal negative control sites due to the diversity of cell types present, however this must be confirmed 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 is observed, the negative control tissue must be deemed invalid and the results obtained with the patient or tissue specimen must also be treated as such.

### Patient Tissue

Patient specimens should be analyzed only once the positive and negative control tissues have been deemed as valid. Negative staining indicates that the antigen was not detected; the use of a panel of antibodies may allow for recognition of false negative results, as negative staining in any one test does not confirm the absence of the antigen in question.

A tissue section stained with hematoxylin and eosin should be used to analyze the morphology of the patient tissue sample, as verified by a qualified pathologist.

## 7. Troubleshooting

1. If the tissue sections wash off the slide, this may be due to:
  - a) If the slides are not positively charged.
  - b) Inadequate drying of the tissue section prior to staining.
  - c) Inadequate neutral-buffering of the formalin used for the fixation process.
  - d) A thick tissue section.
2. If the positive control tissue exhibits negative staining, this may be due to:
  - a) The primary antibody or one of the secondary reagents.
  - b) Improper collection, fixation, or deparaffinization of the tissue section.
3. If the positive control tissue exhibits weaker staining than expected, this may be due to the primary antibody or one of the secondary reagents. Any other positive controls run simultaneously should be analyzed to determine the cause.
4. If non-specific staining occurs, this will have a diffuse appearance and may be due to:
  - a) Improper or suboptimal fixation of tissue sections which may result in sporadic light staining of connective tissue.
  - b) The use of necrotic or degenerated cells. Intact cells should be used for analysis of staining results.

For assistance with all other inquiries, contact GenomeMe® Customer Service at [info@GenomeMe.ca](mailto:info@GenomeMe.ca).

## 8. Limitations

1. This antibody is intended for *in vitro* diagnostic (IVD) use by qualified laboratories only and is not intended for use in flow cytometry.
2. Due to inevitable variability in immunohistochemical procedures and variables, appropriate positive and negative controls must be used and documented, and the results are to be interpreted by a qualified pathologist. Staining must be conducted in a certified, licensed laboratory, under the supervision and responsibility of the qualified pathologist.
3. Improper handling and processing of tissue samples may compromise the validity and/or analysis of the results.
4. GenomeMe® provides prediluted antibodies in a ready-to-use, optimally diluted format for use explicitly as instructed. Improper handling and processing of tissue samples and reagents, and any deviation from the recommended procedures outlined herein, may compromise the validity and/or analysis of the results. Due to the potential for variation in tissue processing and fixation, it may be necessary to adjust incubation time for the primary antibody on specific tissue specimens.
5. GenomeMe® provides concentrated antibodies in a format that requires dilution in the optimized buffer, in the

context of appropriate validation by the user. Any diluent different than that specified in the package insert must also be validated by the user to ensure proper compatibility with the antibody. Once diluted, any deviation from the recommended procedures outlined herein may compromise the validity and/or analysis of the results.

6. This antibody, when used with the appropriate detection systems and accessories, detects antigen(s) that remain intact through the tissue fixation, processing, and sectioning as described herein. Any deviations from these recommended procedures may compromise the validity and/or analysis of the results.
7. The clinical outcome indicated by staining results must be analyzed accurately by the qualified pathologist, and the patient's medical history and other histopathological criteria must be taken into account. The user is responsible for interpretation of the results in the context of the patient.
8. Any documented discrepancies or unexplainable results in controls or tissue specimens should be reported to GenomeMe® Customer Service at [info@GenomeMe.ca](mailto:info@GenomeMe.ca). Patient results are invalid if analysis of the positive and negative control tissues yields results other than those approved and described herein. The Troubleshooting section of this insert may be referred to for unexplained discrepancies in control tissues.
9. The potential for unexpected results in patient tissue specimens cannot be eliminated due to inherent biological variability in the expression of certain antigens.
10. The potential for false positive results in patient tissue specimens cannot be eliminated due to the possibility of non-immunological binding of substrate reaction products or proteins. False positive results may also occur subject to the type of immunostaining technique used, or due to the activity of pseudoperoxidase, endogenous peroxidase, or endogenous biotin.
11. Due to the effect of autoantibodies or natural antibodies, normal sera from an animal source the same as the secondary antisera may result in false negative or false positive results when used in blocking steps.
12. Non-specific staining with horseradish peroxidase may be observed when using tissues containing hepatitis B surface antigen due to the patient's infection with the hepatitis B virus.

## 9. Warnings and Precautions

1. Ensure proper handling procedures are used with all reagents. Always wear laboratory coats, disposable gloves, and other appropriate laboratory equipment when handling reagents.
2. Do not ingest reagents, and avoid contact with eyes and mucous membranes. Wash eyes with copious amounts of water if contact occurs.
3. All incubation times and temperatures must be validated by the user, as must any storage conditions different than those specified in the package insert.
4. Prediluted antibody is provided in a ready-to-use, optimally diluted format, and any further dilution may result in loss of antigen staining.
5. Concentrated antibody requires dilution in the optimized buffer (refer to Materials and Methods), in the context of appropriate validation by the user.
6. Handle tissue sections, patient specimens, and all materials contacting them as biohazardous materials, using the appropriate precautions.
7. To ensure proper stability of the antibody and validity of results, use proper handling of the reagent and avoid microbial contamination.

## 10. References

1. Waalkes S, et al. BMC Cancer. 2010; 10:503. 2. Chen SH, et al. Anticancer Res. 2010; 30:4177-86.