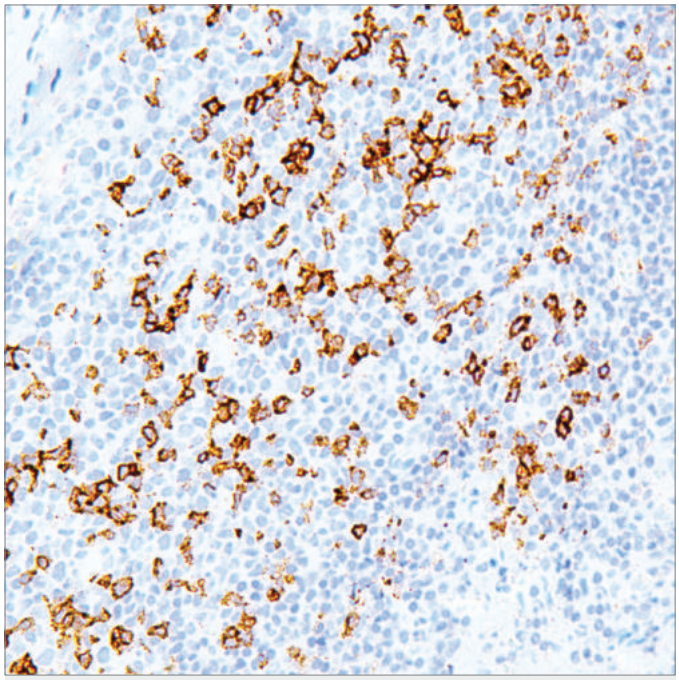
GeneAb™

PD-1

Research Use Only

Clone: IHC001 | **Source:** Mouse Monoclonal | **Positive Control:** Tonsil



GeneAb™ PD-1 (IHC001) on tonsil tissue

Product Information

REF	Description
IHC001-100	0.1 ml Concentrate
IHC001-1	1.0 ml Concentrate
IHC001-7	7.0 ml Pre-dilute, Ready-to-Use
IHC001-PC	Positive control slides, 3 slides/case

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1. Intended Use

The PD-1 (IHC001) antibody is intended for qualiÿed laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-ÿxed, para°n- embedded tissue sections using IHC test methods.

2. Summary and Explanation

Programmed Death 1 (PD-1) is a member of the CD28/CTLA-4 family of T-cell regulators, expressed as a co-receptor on the surface of activated T-cells, B-cells, and macrophages. New studies have suggested that the PD-1/PD-L1 signaling pathway may be linked to anti-tumor immunity, as PD-L1 has been shown to induce apoptosis of activated T-cells or inhibit activity of cytotoxic T-cells. In comparison to CD10 and bcl-6, PD-1 is expressed by fewer B-cells and has therefore been considered a more speciÿc and useful diagnostic marker for angioimmunoblastic T-cell lymphoma. Therapies targeted toward the PD-1 receptor have shown remarkable clinical responses in patients with various types of cancer, including non–small cell lung cancer, melanoma, and renal-cell cancer.

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 (link antibody) which speciÿcally 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

Reagents Provided

Product	Optimized Buffer Composition
Predilute	Antibody Diluent Bu¸ er
Concentrate	Tris Bu¸ er, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide
Recommended working dilution range	1:100 - 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 bu¸ er, to the recommended working dilution range (see Reagents Provided).

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**.

Specimen Collection and Preparation for Analysis

Each tissue section should be ÿxed with 10% neutral bu¸ ered 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 ÿxation time or special processes of speciÿc tissue preparations.

5. Instructions For Use

Recommended Staining Protocols for the PD-1 (IHC001) 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 (Pre-dilute) or dilute antibody 1:100-1:200 (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 validated using Leica® Biosystems' BOND-MAX Autostainer, 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 Bond ER Solution 2 for 30 minutes.

c) Move Peroxide Block step to after Polymer and before Mixed DAB reÿne.

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

Positive Control Tissue

A positive control tissue must be run with each staining procedure, and must be prepared and ỹxed identically to the test sections in order to provide control for all test variables, including ỹxation 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. Tonsil tissue can be used as positive control tissue for the PD-1 (IHC001) 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 anappropriate 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 deỹned herein is not observed, the results obtained must be treated as invalid. The positive control tissue should be used 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 o¸er internal negative control sites due to the diversity of cell types present, however this must be conỹrmed by the user. The components that do not stain should demonstrate the absence of speciỹc staining, and provide an indication of non-speciỹc background staining. If speciỹc staining is observed, the negative control tissue must be deemed invalid and the results obtained must also be treated as such.

Tissue Specimens

Tissue 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 conỹrm the absence of the antigen in question.

7. Troubleshooting

1. If the tissue sections wash o¸ 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-bu¸ering of the formalin used for the ỹxation process

   d) The thickness of the tissue

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, ỹxation, or depara°niza tion 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-speciỹc staining occurs, this will have a di¸use appearance and may be due to:

   a) Improper or suboptimal ỹxation 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**.

8. Limitations

1. Due to inevitable variability in immunohistochemical procedures and variables, appropriate positive and negative controls must be used and documented.

2. Improper handling and processing of tissue samples may compromise the validity and/or analysis of the results.

3. 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 ÿxation, it may be necessary to adjust the incubation time for the primary antibody on speciÿc tissue specimens.

4. GenomeMe® provides concentrated antibodies in a format that requires dilution in the optimized bu¸er, in the context of appropriate validation by the user. Any diluent di¸erent than that speciÿed 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.

5. This antibody, when used with the appropriate detection systems and accessories, detects antigen(s) that remain intact through the tissue ÿxation, processing, and sectioning as described herein. Any deviation from these recommended procedures may compromise the validity and/or analysis of the results.

6. Any documented discrepancies or unexplainable results in controls or tissue specimens should be reported to GenomeMe® Customer Service at **info@GenomeMe.ca**. 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.

7. The potential for unexpected results icannot be eliminated due to inherent biological variability in the expression of certain antigens.

8. The potential for false positive results 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 pseudo-peroxidase, endogenous peroxidase, or endogenous biotin.

9. Due to the e¸ect 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.

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 di¸erent than those speciÿed 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 bu¸er (refer to *Reagents Provided*), in the context of appropriate validation by the user.

6. Handle tissue sections 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

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