

IDH1 R132H

Clone: IHC132

Source: Mouse Monoclonal

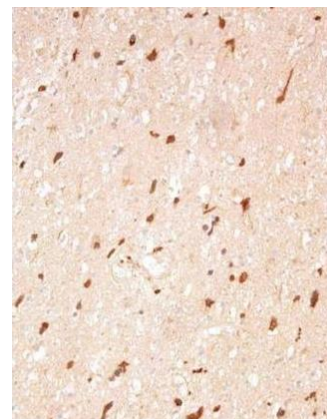
Positive Control: Astrocytoma

Cat. # AO1320100MP, 100 µl

Cat. # AO1321000MP, 1 ml

Intended Use

The IDH1 R132H antibody is for research use to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods.



Human IDH1 R132H on Astrocytoma

Summary and Explanation

Isocitrate Dehydrogenase 1 (IDH1) is a soluble, cytosolic enzyme involved in the TCA metabolic cycle. The most notable mutation in this enzyme, R132H, is clinically indicated in the majority of astrocytomas and oligodendroglial tumors, with the mutation being associated with more favorable prognosis and increased survival in those patients. IDH1 R132H is also useful in the differential diagnosis between anaplastic glioma and glioblastoma.

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 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.

Materials and Methods

Reconstitution, Mixing, Dilution, and Titration

The antibody solvent is Tris Buffer, pH 7.3-7.7, with 1% BSA and <0.1% Sodium Azide. Dilute the concentrated antibody in an appropriate buffer 1:100 – 1:200 to achieve the recommended working dilution range.

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 As One Customer Service at info@asone-int.com

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.

Instructions for Use

Manual Use:

1. Pretreatment: Perform heat-induced epitope retrieval (HIER) at pH 9 for 10-30 mins.
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 diluted 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' Autostainer, VENTANA Staining Protocols.

Leica® Biosystems' Autostainer Standard IHC Protocol F with the following changes:

1. Marker Incubation Time: 30 Minutes
2. Heat-induced epitope retrieval (HIER) is recommended using Bond ER Solution 2 for 30 minutes.
3. Move Peroxide Block step to after Polymer and before Mixed DAB refine.

VENTANA platforms staining protocol:

1. Cell Conditioning: CC1 36 minutes
2. Primary Antibody incubation: 32 minutes
3. Ultrawash
4. Detection: ultraView with DAB

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

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

Astrocytoma tissue can be used as positive control tissue for the IDH1 R132H (IHC132) 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.

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.

Warnings and Precautions

Ensure proper handling procedures are used with all reagents. Always wear laboratory coats, disposable gloves, and other appropriate laboratory equipment when handling reagents.

Do not ingest reagents, and avoid contact with eyes and mucous membranes. Wash eyes with copious amounts of water if contact occurs.

All incubation times and temperatures must be validated by the user, as must any storage conditions different than those specified in the package insert.

Concentrated antibody requires dilution in the optimized buffer (refer to Reagents provided), in the context of appropriate validation by the user.

Handle tissue sections, patient specimens, and all materials contacting them as biohazardous materials, using the appropriate precautions.

To ensure proper stability of the antibody and validity of results, use proper handling of the reagent and avoid microbial contamination.