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Hepa-Ab

XG001

An immunohistochemical kit for the detection of Hepatocellular Carcinoma (HCC) from liver biopsies

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INTENDED USE

Hepa-Ab contains an oligoclonal antibody anti-Squamous Cell Carcinoma antigen (anti-SCCA) variants, providing qualitative and semi-quantitative demonstrations of SCCA variants (SCCA-1, SCCA-2, SCCA-PD) in formalin-fixed, paraffin-embedded human liver tissue sections (1) or cryostat human liver sections.

EXPLANATION

Hepa-Ab is an innovative immunohistochemical kit for the diagnosis of Hepatocellular Carcinoma (HCC) in surgical and fine-needle biopsies based on the detection of SCCA variants (1,2,3). High levels of SCCA variants have been detected in all HCC surgical tumors, with no reactivity detectable in normal human livers (1,2). Over-expression of SCCA variants has indicated the pathological stages of HCC. No correlation due to etiology was found (1,2). Moreover, expression levels of SCCA have been shown to be significantly higher in tumoral tissue than in cirrhotic peritumoral tissue (2,4,5). Hepa-Ab has also been used to determine the expression of SCCA variants in squamous cell carcinomas (6).

PRINCIPLE OF THE TEST

Specific immunostaining is accomplished by localizing the SCCA variants antigens with Hepa-Ab by the use of the avidin-biotin peroxidase (ABC) method (7). Specifically, with this method a biotinylated secondary antibody binds to the primary antibody that is complexed with any SCCA variants located in the tissue. A chromogen solution is then added forming a colored deposit (usually brown) in the presence of the avidin-biotin peroxidase complex, thus revealing the SCCA variants. The sample is counterstained and a coverslip is mounted to ensure best viewing. Results are interpreted using a light microscope. The interpretation of immunostaining results must be complemented with the use of positive and negative controls to ensure correct methodology.

REAGENTS AND MATERIALS PROVIDED

XG001: lyophilized rabbit anti-Squamous Cell Carcinoma antigen (anti-SCCA) variants from a solution containing 20mM phosphate pH 7.2
Reconstitution buffer: 1 mL phosphate buffer saline

STORAGE CONDITIONS

Avoid repeated freeze and thaw cycles
Unopened kit may be stored at 4 °C.
Once resuspended, the antibody may be stored at 4 °C for short-term only.
For long-term storage, store resuspended antibody in aliquots at -20 °C.
The Prepare Working Dilutions only on the day of use.

EXPIRATION DATE

Expiration date printed on the kit indicates limits of stability.

CONTROLS

Positive tissue control: epidermic and follicular area

1. Non-patient tissue containing antigen to be detected
2. Tissue fixed and processed in the same way as patient sample

Objective:

1. Control all steps of the analysis
2. Training of user for the appearance of positive reaction
3. Semiquantitative comparison of the reaction

Negative tissue control: dermic area

1. Tissue that does not express the antigen
2. Tissue that is fixed and processed in the same way as patient sample

Objective:

1. Detects of unintended antibody cross-reactivity
2. Provides the absence of specific immunostaining
3. Provides an indication of non-specific background staining



INSTRUCTIONS FOR USE

REAGENTS PREPARATION

Resuspend Hepa-Ab antibody by adding 1,0 mL of the Reconstitution Buffer provided and gently agitate the vial with its top securely attached in order to dissolve any antibody that may be under the cap. Resuspension should occur at least 1 hour before use and vial should be allowed to sit at 4°C in order to dissolve the lyophil completely and to secure its equal distribution in the buffer

PROTOCOL

1. De-Paraffinate tissue sections by incubation in xylene for 30 minutes
2. Hydrate by placing in 100%, 95%, 70%, 50% ethanol
3. Wash with deionized water
4. Inactivate the endogenous peroxidase by incubation for 10 minutes in 3% hydrogen peroxide in PBS.
5. Wash with PBS containing 0.1% Tween.
6. Perform antigen unmasking by placing the slides with samples in a 0.01 M citrate buffer pH 6 bath and heating in microwave. We suggest performing 3 cycles of 5 minutes each at 750 Watt*.
7. Wash with PBS containing 0.1% Tween.
8. Block slides with animal serum taken from the same secondary antibody animal species. Incubate for 10 minutes at room temperature in a humidified chamber.
9. Remove residual liquid.
10. Incubate slides in a humidified chamber for 1 hour at room temperature with a Hepa-Ab dilution range of 1:5 – 1:10 in PBS containing 0.1% Tween*. Special attention should be taken in order to avoid drying out of the sample.
11. Wash with PBS containing 0.1% Tween
12. Incubate with biotinylated secondary antibody for 10 min at room temperature in a humidified chamber diluted as described by manufacturer
13. Wash with PBS containing 0.1% Tween.
14. Incubate with ABC complex for 10 min at room temperature
15. Wash with PBS containing 0.1% Tween.
16. Incubate with chromogen solution (DAB) for maximum 5 minutes. Viewing of the sample under the microscope may help determine a correct incubation time to avoid overstaining *
17. Wash with deionized water

18. Counterstain with hematoxylin solution for 1 minute[§]
19. Wash with warm tap water
20. Dehydrate slides in 80%, 90% and 2x 100% ethanol
21. Immerse in 2 changes of xylene
22. Mount cover slips on the slides and examine under a light microscope

(*) These are guidelines only; each lab should determine optimal dilution and incubation time.

(§) When interpreting an IHC result, the morphology of each tissue sample should be examined by hematoxylin and eosin (H&E) staining.

SUMMARY OF EXPECTED IMMUNOSTAINING

Hepa-Ab stains predominantly the cytoplasm of the liver cancer cells (1, 2).

INTERPRETATION

The immunostaining intensity will reflect not only the effects of tissue preparation, but especially antigen concentration. An intense immunostaining indicates a relatively high concentration of HCC molecular marker, while lighter immunostaining will be indicative of a lower concentration. It should be helpful to score the percentage of stained cells in each specimen as follows (1):

- Score 0: Denotes negative staining
- Score 1: positivity in 1-30%
- Score 2: positivity in 31-50%
- Score 3: positivity in more than 50%

TROUBLESHOOTING

Negative/Low staining on positive tissue

- Procedure may not have been run properly
- Procedure steps were omitted or performed in the wrong order
- Deparaffinization was not complete
- Degradation of antigens
- The primary antibody may be too diluted
- The detection system reagents may be too diluted
- Incubation times of the detection system reagents may be too short
- Counterstain or coverslip mounting is incompatible with chromogen
- Antigen unmasking may not have been done properly
- Tissue may be not properly prepared or may be overfixed



Non-specific background staining

- Protein blocking step was ineffective
- Endogenous enzyme activity
- Deparaffinization was not complete
- Reagent dried on tissue during immunostaining
- Primary antibody or detection system reagents are too concentrated or incubation times are too long

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