REFERENCES

TRADEMARKS
Clearmount™, Digest-All™, HistoGrip™, Histomount™, and Histostain®, and Zymed® are trademarks of Invitrogen Corporation.
SPECIFICITY
Mouse anti-Hepatitis C Virus (clone: TORDJI-22) reacts specifically with hepatitis C virus. Mouse anti-Hepatitis C Virus (clone: TORDJI-22) is specific for the non-structural region of HCV (NS3-NS4). A granular staining pattern is observed in the cytoplasm of infected cells. No cross-reactivity with hepatitis B surface antigen or hepatitis A has been detected.

MATERIALS REQUIRED BUT NOT PROVIDED
Reagent Invitrogen Cat. No.
1. HistoGrip™ 00-8050
2. Super PAP Pen 00-8899
3. Purified mouse IgG-kappa immunoglobulin (non-immune) 08-6599
4. Antibody Diluent 00-3118
5. PBS (0.01 M PBS) 00-3000
6. Digest-All™ 3 (Pepsin) 00-3009
7. Mayer’s hematoxylin 00-8011
8. LAB-SA (Histostain®-Plus, and Cap-Plus™ kits) for detecting murine primary antibodies on human samples (other kits are available, call for info.)

Table 1. Invitrogen Immunohistochemistry Detection Kits.

<table>
<thead>
<tr>
<th>Detection Kits</th>
<th>Enzyme</th>
<th>Chromagen</th>
<th>Size</th>
<th>1° Ab Reactivity</th>
<th>Invitrogen Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histostain®-Plus</td>
<td>HRP</td>
<td>AEC</td>
<td>15 mL</td>
<td>Broad Spectrum*</td>
<td>85-9943</td>
</tr>
<tr>
<td>Histostain®-Plus</td>
<td>HRP</td>
<td>AEC</td>
<td>15 mL</td>
<td>Mouse</td>
<td>85-6543</td>
</tr>
<tr>
<td>Histostain®-Plus</td>
<td>HRP</td>
<td>DAB</td>
<td>15 mL</td>
<td>Broad Spectrum*</td>
<td>85-9643</td>
</tr>
<tr>
<td>Histostain®-Plus</td>
<td>HRP</td>
<td>DAB</td>
<td>15 mL</td>
<td>Mouse</td>
<td>85-9143</td>
</tr>
<tr>
<td>Histostain®-Plus</td>
<td>HRP</td>
<td>---</td>
<td>60 mL</td>
<td>Broad Spectrum*</td>
<td>85-8943</td>
</tr>
<tr>
<td>Histostain®-Plus</td>
<td>AP</td>
<td>Fast-Red</td>
<td>15 mL</td>
<td>Broad Spectrum*</td>
<td>85-9942</td>
</tr>
<tr>
<td>Histostain®-Plus</td>
<td>AP</td>
<td>---</td>
<td>60 mL</td>
<td>Broad Spectrum*</td>
<td>85-8942</td>
</tr>
<tr>
<td>Cap-Plus™ Detection Kit</td>
<td>HRP</td>
<td>DAB</td>
<td>110 mL</td>
<td>Broad Spectrum*</td>
<td>87-8143</td>
</tr>
<tr>
<td>Cap-Plus™ Buffer Kit</td>
<td>For use with Cap-Plus™ Detection Kit</td>
<td></td>
<td></td>
<td></td>
<td>87-0003</td>
</tr>
</tbody>
</table>

* detects mouse, rabbit, rat, and guinea pig primary antibodies

10. Chromogen/substrate (if not included with detection kit): Single Solution AEC (aminoethyl carbazole, Invitrogen Cat. No. 00-1111), or DAB (3,3’-diaminobenzidine, 00-2014), or Fast-Red (00-2234).
11. Mounting solution: Histomount™ for DAB (Invitrogen Cat. No. 00-8030), GVA (for AEC or Fast-Red) 00-8000, or Clearmount™ for AEC, DAB, or Fast-Red.

Also: Coverslips, humidifying chamber, microscope, microscope slides, timer, staining jars, deparaffinizing and rehydrating reagents.
INSTRUCTIONS FOR USE

Invitrogen’s Mouse anti-Hepatitis C Virus (clone: TORDJI-22) may be diluted according to the dilution table below (Table 3) when using the specified Histostain® (LAB-SA) and Cap-Plus™ detection systems. More concentrated solutions may be necessary for PAP and ABC methods. More or less concentrated solutions may be necessary for LAB-SA kits not provided by Invitrogen.

Table 2. Immunohistostaining Requirements

<table>
<thead>
<tr>
<th>Epitope Retrieval</th>
<th>Enzyme Digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Required</td>
<td>Digest All™ 3 Required (catalog number 00-3009)</td>
</tr>
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Table 3. Dilution Table

<table>
<thead>
<tr>
<th>Invitrogen Kit</th>
<th>Dilution*</th>
<th>Incubation Time</th>
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<tr>
<td>Histostain®-SP or SAP Kits</td>
<td>1:50 – 1:100</td>
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<td>Cap-Plus™ Kits</td>
<td>1:100 – 1:200</td>
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*These recommendations are for guidance only. Each end-user laboratory should determine optimal dilutions and procedures. Improperly diluted primary antibodies may result in non-specific or false-negative staining if solutions are too strong or too weak, respectively.

QUALITY CONTROL

Differences in tissue processing and technical procedures in the user’s laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures. Consult the quality control guidelines of the following: Special Report: Quality control in immunohistochemistry [13], the proposed NCCLS guideline for IHC [14], and the College of American Pathologists (CAP) Certification Program for Immunohistochemistry [15].

Positive Tissue Control: External positive control materials should be fresh autopsy/biopsy/surgical specimens fixed, processed and embedded as soon as possible in the same manner as the test sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive external tissue control for each set of test conditions should be included in each staining run.

The tissues used for the external positive control materials should be selected from sample specimens with well-characterized low levels of the positive target activity that gives weak positive staining. The low level of positivity for external positive controls is designed to ensure detection of subtle changes in the primary antibody sensitivity from instability or problems with the IHC methodology. Specimens processed differently from the test sample(s) validate reagent performance only and do not verify tissue preparation.

Skin or prostate gland may be useful as a source of positive control tissue.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control: Use a non-specific negative tissue control (known to be negative for HCV staining) fixed, processed, and embedded in a manner identical to the sample(s) with each staining run. This will verify the specificity of the IHC primary antibody for demonstration of the target antigen, and provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can be used by the laboratorian as internal negative control sites to verify the test’s performance specifications.

If specific staining (false positive staining) occurs in the negative tissue control, results with the sample specimens should be considered invalid.

REAGENT PREPARATION

This antibody is concentrated and requires dilution before use. Dilute antibody with Antibody Diluent (Invitrogen Cat. No. 00-3118 or 00-3218). Each laboratory has to determine optimal dilutions for their own applications.

PRODUCT SPECIFIC LIMITATIONS

Requires enzyme pretreatment (Digest-All™ 3 Pepsin) of FFPE tissue sections for proper staining. Failure to pretreat FFPE tissue sections may result in reduced staining for false negative results.

STORAGE

Store at 2-8 °C. Fresh dilutions of the antibody should be made prior to use. Unused portions of antibody preparations should be discarded at the end of the day.

This antibody is suitable for use until the expiration date on the label when stored at 2-8°C. Do not use after expiration date on vial. If product is stored under any conditions other than those specified in this package insert, those storage conditions must be verified by the user. This antibody has not been prepared by aseptic techniques.

Reagents should not be used if deterioration or substantial loss of activity is evident. The normal appearance of this product is a slightly yellowish liquid free of particulate matter.

Positive and negative controls should be run simultaneously with all sample specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and if a problem with the antibody is suspected, Invitrogen Laboratories, Inc., (800) 955-6288 should be contacted immediately.

SPECIMEN PREPARATION

Paraffin-embedded Sections: Tissue fixed in neutral buffered formalin prior to paraffin-embedding is suitable for use. Other fixation methods may be useful but have not been tested. Tissues sections need to be deparaffinized and rehydrated before staining.

Properly fixed and embedded tissues expressing the target antigen should be stored in a cool place. Consult references 10, 11, and 12 for further details on specimen preparation.

Treatment of Tissue Sections Prior to Staining:

For optimal staining, pepsin pretreatment is recommended. Preheat the solution to 37°C and incubate the tissue sections for 10 minutes at 37°C.

SAFETY & PRECAUTIONS

1. For research use only.
2. Take appropriate precautions when handling reagents. Use disposable gloves, coat, and safety glasses when handling suspected carcinogens.
3. Do not use this antibody beyond expiration date for prescribed storage method.
4. After use, store as specified. Any storage conditions other than those specified in the package insert must be validated by the user.
5. Do not smoke, eat or drink in areas where specimens or this antibody are being handled.
6. Avoid contact of eyes and mucous membranes with this antibody. If this antibody comes in contact with sensitive areas, wash with generous amounts of water.
7. The sodium azide (Na₃N₃) 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. The concentration of sodium azide in this antibody is 0.05%. This product does not meet the Occupational Safety & Health Administration (OSHA) criteria for a hazardous substance, and therefore no Material Safety Data Sheet (MSDS) is required.
8. Consult Federal, State or local regulations for disposal of any potentially toxic components.
9. Sample specimens and all materials coming into contact with them should be handled as if capable of transmitting infection, and disposed of with proper precautions. Never pipette by mouth, and avoid contact of this antibody and specimens with skin and mucous membranes.
10. Minimize microbial contamination of this antibody or an increase in non-specific staining may occur.
11. Use of incubation times or temperatures other than what is specified may give erroneous results. The user must validate any such change.

Table 4. Immunohistostaining Requirements

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Table 4: The Purpose of Daily Quality Control

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<tr>
<th>Tissue: Fixed &amp; Processed Like Sample</th>
<th>Specific Antibody &amp; Secondary Antibody</th>
<th>Nonspecific Antibody* or Buffer with Same Secondary Antibody as Used with Specific Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control: tissue or cells containing target antigen to be detected (could be located in sample tissue). The ideal control is weakly positive staining tissue to be most sensitive to antibody degradation.</td>
<td>Controls all steps of the analysis. Validates reagent and procedures used for staining.</td>
<td>Detection of non-specific background staining.</td>
</tr>
<tr>
<td>Negative Control: Tissues or cells expected to be negative (could be located in sample tissue or positive control tissue)</td>
<td>Detection of unintended antibody cross-reactivity to cells/cellular components.</td>
<td>Detection of non-specific background staining</td>
</tr>
<tr>
<td>Sample Tissue</td>
<td>Detection of specific staining.</td>
<td>Detection of non-specific background staining</td>
</tr>
</tbody>
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* = Same source and type as the specific antibody but not directed against the same target antigen. To detect non-specific antibody binding, e.g., binding of Fc portion of antibody by the tissue.

Refer to Summary and Explanation, Limitations, and Performance Characteristics for specific information regarding immunoreactivity.

Any controversial staining should be reported to Zymed Laboratories, Inc.’s Technical Service Department.

LIMITATIONS
1. Immunohistochemistry is a multistep process that consists of specialized training in the selection 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. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of sample results under these circumstances.
5. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
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. Contact Invitrogen at (800) 955-6288 with documented unexpected reaction(s).
7. Normal/non-immune serum from the same animal source as secondary antiserum used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
8. False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome c), or endogenous biotin (e.g. liver, breast, brain, kidney) (depending on the type of immunostain used).

Nonspecific Negative Reagent Control: Use a nonspecific negative reagent (isotype control) in place of the primary antibody with a section of each sample specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. Ideally, a negative reagent control contains mouse IgG, protein, purified from ascites fluid, that exhibits no specific reactivity with human tissues. Dilute a mouse IgG, antibody (Invitrogen Cat. No. 02-6100) to the same immunoglobulin or protein concentration as the diluted primary antibody using the identical diluent or use purified mouse IgG, immunoglobulin (Invitrogen Cat. No. 08-6599). Diluent alone may be used as a less desirable alternative to the previously described negative reagent control.

The incubation period for the negative reagent control should correspond to that of the primary antibody. Use a negative reagent control in place of the primary antibody with a section of each sample specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. To prepare a negative reagent control, dilute an immunoglobulin fraction of normal/non-immune serum to the same protein concentration as the diluted primary antibody using the identical diluent. When panels of several antibodies are used on serial sections, the negatively staining areas of one slide may serve as a negative/nonspecific binding background control for other antibodies.

To differentiate endogenous enzyme activity from specific immunoreactivity, additional sample tissues may be stained exclusively with substrate-chromogen. To differentiate endogenous biotin activity or nonspecific binding of enzymes from specific immunoreactivity, additional sample tissues may be stained exclusively with enzyme-streptavidin conjugate and substrate-chromogen.

ASSAY VERIFICATION
Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody’s specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative tissues. Refer to the quality control procedure outlined in this product insert and to the quality control recommendations of the CAP Certification Program for Immunohistochemistry and/or the NCCCLS IHC guideline. These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters.

TROUBLESHOOTING
Refer to the troubleshooting guide in the package insert of the Invitrogen detection kit being used or call Invitrogen’s Technical Service Department at (800) 955-6288.

INTERPRETATION OF STAINING
Positive Tissue Control: The positive tissue control stained with primary antibody should be examined first to ascertain that all reagents are functioning properly. The presence of positive stain in the cell cytoplasm is indicative of positive reactivity.

The color of the reaction product may vary if substrate chromogens other than those stated are used. Refer to substrate package inserts for expected color reactions. Further, metachromasia may be observed in variations of the method of staining.

It is recommended that hematoxylin be used for counterstaining. Counterstaining will result in a pale to dark blue coloration of the cell nuclei depending on the incubation time and potency of the hematoxylin used. Excessive or incomplete counterstaining may compromise proper interpretation of results.

Negative Tissue Control: The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen. The absence of specific staining in the negative tissue control confirms the lack of antibody cross-reactivity to cells/cellular components. If specific staining (false positive staining) occurs in the negative external tissue control, results with the sample specimen should be considered invalid.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Sample Tissue: Examine sample specimens, stained with primary antibody, last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.