Novocastra™ Lyophilized Mouse Monoclonal Antibody
Hepatitis C virus (NS3)

Product Code: NCL-HCV-NS3

Intended Use
FOR RESEARCH USE ONLY.

Specificity
Non-structural protein 3 (NS3) of human hepatitis C virus. NCL-HCV-NS3 recognises an epitope stable to routine processing and exhibits minimal crossreactivity with normal or diseased liver, although non-specific staining has been observed in heavily immunosuppressed individuals with cholestasis.

Clone
MMM33

Ig Class
IgG2b

Antigen Used for Immunizations
Prokaryotic recombinant protein corresponding to non-structural protein 3 of hepatitis C virus, strain 1b.

Hybridoma Partner
Mouse myeloma (p3-NS1-Ag4-1).

Preparation
Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.

Effective on Frozen Tissue
Yes. Acetone/chloroform (1:1)-fixed frozen sections or cryostat sections of paraformaldehyde/sucrose fixed tissue (followed by the high temperature antigen unmasking technique).

Effective on Paraffin Wax Embedded Tissue
Yes (using the high temperature antigen unmasking technique: see overleaf).

Recommendations on Use
Immunohistochemistry: Typical working dilution 1:25–1:50. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Five-step peroxidase, anti-peroxidase detection system: see overleaf. Alternatively, other sensitive detection systems which exclude the use of biotin, may be utilised. Western Blotting: Not evaluated.

Positive Controls
Immunohistochemistry: Liver acutely infected with hepatitis C virus.

Staining Pattern
Infected hepatocytes; cytoplasmic.

Storage and Stability
Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.

General Overview
Non-structural protein 3 (NS3) of hepatitis C virus (HCV) is a protein of 67 to 70 kD that contains at least three enzymatic activities; a protease, a serine protease and an RNA helicase. NS3 is produced as part of a polyprotein of about 3000 amino acids which is encoded by the viral genome. This polyprotein undergoes proteolytic processing by a combination of cellular and viral proteases, including those provided by NS3, to yield all of the mature viral proteins. The RNA helicase activity of NS3 is thought to be involved in viral replication.

General References
Instructions for Use

Immunohistochemical Demonstration of Hepatitis C Virus (NS3 Antigen) in Fixed Liver Sections

1. Cut and mount sections on slides coated with APES.
2. Place air-dried frozen sections or deparaffinized, rehydrated paraffin sections in 1.5% hydrogen peroxide/methanol for 10 minutes.
3. Wash sections in running tap water for 5 minutes.
4. Bring 1600 mL 0.01 M sodium citrate buffer (pH 6.0) to the boil in a Prestige stainless steel pressure cooker, using a hot plate. Cover but do not lock lid.
5. Position slides into metal staining racks and lower into pressure cooker ensuring slides are well immersed in citrate buffer. Lock lid and bring up to temperature and pressure.
6. When maximum temperature has been reached, incubate sections for 1 minute.
7. Remove pressure cooker from heat source and run under cold water with lid on. When the small valve sinks open lid and remove slides and then place immediately into distilled water. DO NOT OPEN LID UNTIL THE SMALL VALVE SINKS.
8. Wash sections in running water for 5 minutes.
9. Wash sections in Tris-Buffered Saline buffer for 2 x 5 minutes.
10. Place sections in diluted normal serum for 20 minutes.
11. Cover sections with primary antibody. (The optimal dilution of the antibody, incubation time and incubation temperature should be determined by the individual laboratory.)
12. Wash in TBS buffer for 2 x 5 minutes.
13. Incubate sections in appropriately diluted anti-mouse peroxidase-conjugated link antibody for 30 minutes.
14. Wash in TBS buffer for 2 x 5 minutes.
15. Incubate sections in an appropriately diluted mouse PAP for 30 minutes.
16. Wash in TBS buffer for 2 x 5 minutes.
17. Repeat steps 13 to 15.
18. Incubate slides in DAB.
19. Wash in water for 2 x 5 minutes.
20. Counterstain with hematoxylin (if required), dehydrate, coverslip and mount.

Materials
1. APES (3-aminopropyltriethoxysilane) (Catalogue No A3648) Sigma Immunochemicals.
   To avoid sections becoming detached, sections should be mounted on Leica Microscope slides or APES treated slides, then dried at 37 °C overnight followed by heating at 56 °C for 60 minutes.
2. 0.01 M CITRATE BUFFER (pH 6.0)
   Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

SAFETY NOTE
To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER’S INSTRUCTIONS.