Novocastra™ Lyophilized Mouse Monoclonal Antibody Herpes simplex virus (type 1)

Product Code: NCL-HSV-1

Intended Use
FOR RESEARCH USE ONLY.

Specificity
NCL-HSV-1 reacts with herpes simplex virus type 1. Crossreaction has been observed with some strains of poliovirus type 2 and cytomegalovirus (CMV) laboratory strain AD169 but not with primary tissue culture isolates of CMV. NCL-HSV-1 does not crossreact with tissue culture isolates of respiratory syncytial virus, influenza virus types A and B, parainfluenza virus types 1, 2, 3 and 4b, adenovirus, herpes simplex virus type 2, varicella-zoster virus, mumps virus, measles virus, ECHO virus 19, coxsackie B4 virus, poliovirus types 1 and 3 or negative tissue culture cells used in routine virus isolation.

Clone
20.7.1

Ig Class
IgG1

Antigen Used for Immunizations
Herpes simplex virus type 1 (Stoker strain).

Hybridoma Partner
Mouse myeloma (JK Ag8.653).

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
Not evaluated.

Effective on Paraffin Wax Embedded Tissue
Yes

Recommendations on Use
Immunohistochemistry: Typical working dilution 1:25–1:50. Trypsin digestion of paraffin sections is recommended. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Indirect immunofluorescence: Typical working dilution 1:25–1:50. See overleaf for protocol. Read using immersion oil eg Cargille type FF (Product No. 12612). Western Blotting: Not evaluated.

Positive Controls
Immunohistochemistry: Formalin-fixed, paraffin-embedded herpes simplex virus type 1-infected tissue.
Immunofluorescence: Acetone-fixed Hep 2 cells infected with herpes simplex virus type 1.

Staining Pattern
Nuclear and 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
Herpes simplex type 1 (HSV-1) belongs to a family that includes HSV-2, Epstein-Barr virus (EBV) and Varicella zoster (chicken pox) virus amongst others. HSV-1 and HSV-2 are extremely difficult to distinguish from each other. Members of this family have a characteristic virion structure. The double stranded DNA genome is contained within an icosahedral capsid embedded in a proteinaceous layer ( tegument ) and surrounded by a lipid envelope, derived from the nuclear membrane of the last host, which is decorated with virus-specific glycoproteins spikes. These viruses are capable of entering a latent phase where the host shows no visible sign of infection and levels of infectious agent become very low. During the latent phase the viral DNA is integrated into the genome of the host cell.

General References
Instructions for Use

Description of Methods for Use of Antiviral Antibodies in Indirect Immunofluorescence

Reagents
1. Acetone-fixed cells infected with appropriate virus (positive control).
2. Acetone-fixed uninfected cells (negative control).
3. Appropriate antibody at dilutions for titration.
4. Secondary FITC-conjugated antibody diluted 1:100 in counterstain (Evans blue 0.0005% w/v in phosphate buffered saline).
5. Appropriate mountant for reading slides—see data sheet.

Equipment
Fluorescence microscope, dark humid slide incubation tray, 37 °C incubator.

Procedures
1. Allow slides to reach 25 °C before starting.
2. Apply Novocastra antibody at appropriate dilution (20 µL/spot).
3. Incubate for 30 minutes at 37 °C in a dark, humid slide incubation tray.
4. Rinse 3 x 5 minutes in phosphate buffered saline (PBS) (pH 7.4).
5. Air dry.
6. Apply diluted FITC-conjugated antibody (as described in REAGENTS, point 4).
7. Incubate for 30 minutes at 37 °C in a dark, humid slide incubation tray.
8. Rinse 3 x 5 minutes in PBS (pH 7.4).
9. Rinse slides for 1 minute in distilled water.
10. Air dry.
11. Read under oil using 50x oil objective (see data sheet for recommendation on particular mounting medium eg Cargille immersion oil, Biosoft Flukeep etc).
Instructions for Use

Trypsin Digestion for Immunohistochemical Demonstration on Paraffin Sections

1. Preheat the following to 37 °C using a water bath:
   (i) 200 mL of TBS
   (ii) 200 mL of distilled water.
2. Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium chloride in the 200 mL of TBS.
3. Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.
4. Place rehydrated paraffin sections in the distilled water to preheat the sections to 37 °C for a minimum of 5 minutes.
5. Incubate sections in Trypsin solution at 37 °C. The time required will depend on the antibody and tissue, however, 30 minutes is usually sufficient.
6. Rinse sections in running tap water.
7. Proceed with immunohistochemistry protocol.

Reagents Required but not Supplied

50 mM Tris-buffered saline
Trypsin 250: Difco order code 0152–13 (available from Becton Dickinson).
Calcium chloride
1 M Sodium Hydroxide

* Trypsin containing chymotrypsin should always be used. The enzyme activities can vary from a supplier and between batches. Such variations may affect the incubation time required.