Novocastra™ Lyophilized Mouse Monoclonal Antibody Respiratory Syncytial Virus

Product Code: NCL-RSV3

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Respiratory syncytial virus phosphoprotein, fusion protein and nuclear protein. NCL-RSV3 detects both subtypes of human respiratory syncytial virus A and B. NCL-RSV3 does not crossreact with tissue culture isolates of influenza virus types A and B, parainfluenza virus types 1, 2, 3 and 4b, adenovirus, herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, mumps virus, measles virus, ECHOvirus 19, coxsackie B4 virus, poliovirus types 1, 2 and 3 or negative tissue culture cells used in routine virus isolation.
Clone	5H5N, 2G122, 5A6 and 1C3.
Ig Class	5H5N; IgG1 2G122; IgG1 5A6; IgG2a 1C3; IgG2a
Antigen Used for Immunizations	Respiratory syncytial virus strain A2.
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
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:200–1:400. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Indirect immunofluorescence: Typical working dilution 1:10. See overleaf for protocol. Read using immersion oil eg Cargille type FF (Product No. 12612). Western Blotting: Not recommended.
Positive Controls	Immunohistochemistry: Formalin-fixed, paraffin-embedded RSV infected lung. Indirect immunofluorescence: Acetone-fixed HeLa cells infected with RSV-A2.
Staining Pattern	RSV-infected epithelial cell membranes and cytoplasm.
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	Respiratory syncytial virus (RSV) belongs to the Pneumovirus genus of the Paramyxoviridae family. The RSV genome consists of a single stranded antisense RNA molecule of 15,200 nucleotides that encodes 11 proteins. In addition to the RNA genome the nucleocapsid contains three proteins; the RNA polymerase which makes complementary copies of the genome that serve as messenger RNAs, and the nucleoprotein and phosphoprotein which are essential for transcriptional activity. Other proteins encoded by the viral genome include the non structural proteins NS1 and NS2, a small hydrophobic protein of unknown function, M2-1 and M2-2 which are involved in viral transcription, and the fusion protein which is required for the infectivity of the virus.
General References	Wright C, Oliver K C, Fenwick F, et al.. Journal of Pathology. 182: 238–244 (1997). Routledge E C, McQuillan J, Samson A C R, et al.. Journal of Medical Virology. 15: 305–320 (1985).

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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 Fluokeep etc).

BIOSYSTEMS

Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
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.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.