AQUATIC Diagnostics Ltd

Anti-Nodavirus monoclonal antibody

Product no: P09

Product Information

Product Description

The monoclonal antibody (Mab) against Nodavirus is specific for this virus. The MAb has been tested by ELISA with Betanodavirus isolated from four different fish species, including European Sea Bass (Dicentrarchus labrax), Atlantic cod (Gadus gadus), halibut (Hippoglossus hippoglossus) and Striped Jack (Pseudocaranx dentex). The Mab reacted with Nodavirus isolated from European Sea Bass that has been shown to belong to the RGNNV (red spotted grouper nervous necrosis virus) cluster (Thiéry et al. 1999). It also reacted against Nodavirus isolated from Atlantic cod and halibut that have been shown to belong to the BFNNV (Barfin flounder nervous necrosis virus) cluster (Grotmol et al. 2000; Starkey et al., 2000; Starkey et al. 2001). The Mab did not react with SJNNV (striped jack nervous necrosis virus). SJNNV has been shown to be phylogenetically quite different to the other Nodavirus clusters (Nishizawa, 1997).

The specificity of the Mab has been tested against a range of viral pathogens that infect fish, including Infectious Salmon Anaemia Virus (ISAV), Infectious Pancreatic Necrosis Virus (IPNV), Pancreas Disease Virus and Sleeping Disease Virus, as well as the cell lines CHSE-214, SSN-1 and SHK-1. It has also been tested against a selection of bacteria found in the aquatic environment. No cross-reaction was found with any of the pathogens or cell lines tested.
The Mab is of an IgG2a isotype.

Use of product

The Mab is recommended for use in immunohistochemistry (IHC), but can also be used in IFAT. The optimal conditions for use of this product vary depending on the procedure used. The user must determine the suitability of the product for a particular procedure. This product is for *in vitro* use only.

Vial Contents

Each vial contains 200 µg of lyophilised protein prepared from bovine-free culture medium and contains no animal-derived stabilisers. This is sufficient for between 100-200 tests depending on the area of tissue to be screened in IHC.

The product should be reconstituted as follows:

- Add 1 ml of phosphate buffered saline (PBS) (see buffers) to the vial, then transfer the contents of the vial and store in aliquots. Dilute 1/10 in PBS containing bovine serum albumin (BSA) before use.

Storage

Store at -20°C or below prior to reconstitution. For prolonged storage, the Mab solution should be stored at -20°C, or below. Repeated freeze thawing of the product should be avoided.

Suggested protocol for the detection of Nodavirus in fixed tissue sections by immunohistochemistry

This procedure has been developed to work on tissues fixed in 10%

buffered formalin for 24 hours. Individual protocols may have to be developed depending upon the tissue examined, fixation etc.

Procedure (wear gloves)

- Prepare paraffin-embedded tissue sections.
- Dewax and rehydrate sections in xylene (2 x 5min), 100% ethanol (5 min), 70% ethanol (3 min), then rinse in distilled water.
- Place slides in a humid chamber.
- Keep sections moist at all times - do not allow them to dry out.
- Mark rings around the tissue sections using a wax PAP pen.
- Block endogenous peroxidase activity by incubating the slides for 10 min at room temperature (»22°C) with H_2O_2 in methanol (see buffers).
- Wash the slides three times with PBS.
- Block non-specific binding sites with normal goat serum diluted 1/10 in TBS for 10 min at room temperature.
- Pour off the serum and remove excess serum tapping the slide edges on a paper towel.
- Place 50-100 µl of reconstituted anti-Nodavirus Mab onto the tissue sections (the volume added will depend on the size of sample to be covered) and incubate for 60 min at room temperature in a humid chamber.
- Use appropriate controls i.e. known positive tissue as a positive control and uninfected tissue as a negative control; these should both be incubated with the reconstituted Mab and PBS separately.
- Wash slides three times with PBS.

- Biotin/streptavidin amplification is applied either using a commercially available ABC amplification kit or as follows: developed depending upon the tissue examined, fixation etc.
- Add goat anti-mouse IgG biotin conjugate (1/10 in PBS, 1% w/v BSA) to the slides for 30 min
- Wash slides three times with PBS
- Add streptavadin–horseradish peroxidase (1/100 in PBS, 1% BSA) to the slides for 30 min
- Wash slides three times with PBS
- To visualise the reaction, incubate the slides for 10 min with DAB solution (see buffers) or with a commercially available Tetramethylbenzidine dihydrochloride staining kit following the manufactures instructions.
- If stained with DAB stop the reaction by immersing the slides in tap water and counter-stain them with haematoxylin for 3-4 min.
- Rinse in tap water for 10 minutes.
- Dehydrate the slides in 70% ethanol (3 min), 100% ethanol (5 min), xylene (2 x 5 min)
- Mount the slides with Pertex and leave in fume cupboard to set.
- Examine tissue under a light microscope –infected tissue appears golden brown in colour when stained with DAB (Lai *et al.,* 2001).

Buffers

Phosphate buffered saline (PBS)

0.02M Phosphate, 0.15M NaCl	pH adjusted to 7.2 with HCl
$NaH_2PO_4.2H_2O$	0.876g/l
$Na_2HPO_4.2H_2O$	2.56g/l
NaCl	8.77g/l

Tris buffered saline (TBS)

Trisma base	2.42g
NaCl	29.24g

Dissolve in approximately 900 ml distilled water, adjust pH to 7.2 using HCl and make up to 1 litre

3% (v/v) Hydrogen peroxide in methanol
Add 1ml H_2O_2 (30% v/v solution) to 9 ml methanol

3,3'-Diaminobenzidinetetrahydrochloride (DAB)

Dissolve one 10mg tablet DAB in 6.67mls TBS
Place 0.5 ml aliquots of the solution into bijoux bottles, store at -20°C. For use add 5mls TBS and 0.1ml 1 % H_2O_2 to 0.5 ml aliquot
NB. DAB is a possible carcinogen

References

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Thiéry R, Arnauld C and Delsert C (1999) Two isolates of sea bass, Dicentrarchus labrax L., nervous necrosis virus with distinct genomes. *Journal of Fish Diseases* **22**:201-207

Certificate of Analysis

Anti-Nodavirus monoclonal antibody

Product no.

Batch no.

Date of expiry

Activity in IHC
Cells infected with the virus appear golden brown in colour when stained with DAB.

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