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Anti- *Photobacterium damselae* subsp. *piscicida* **monoclonal antibody**

Product no: P02

Product Information

Product Description

The monoclonal antibody (MAb) against *Photobacterium damselae* subsp. *piscicida* is species-specific. The specificity of the MAb has been tested against a range of bacterial pathogens which infect fish, including a variety of *Vibrio* species and *Photobacterium damselae* subsp. *damselae*, and also a selection of bacteria found in the aquatic environment (Bakopoulos *et al*. 1997). The Mab is of an IgG2A isotype.

Use of Product

The Mab is recommended for use in immunohistochemistry (IHC). 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 into 9 ml of PBS so that the total volume equals 10 ml.

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Certificate of Analysis

Anti- *Photobacterium damselae* subsp. *piscicida* **monoclonal antibody**

Product no.

Batch no.

Date of expiry

Activity in IHC

The bacterium appears golden brown in colour when the Mab is used at the working dilution described in the protocol.

Relevant references

Bakopoulos, V., Adams, A . & Richards, R.H. (1997) The production and characterisation of monoclonal antibodies against the fish pathogen *Pasteurella piscicida*. Journal of Fish Diseases, 20, 307-315.

Adams, A. & Marin de Mateo, M. Immunohistochemical detection of fish pathogens. Techniques in Fish Immunology - 3. Edited by J.S. Stolen, T.C. Fletcher, S.L. Kaattari, A.F. Rowley , Chapter 14, pp 133-144. (1994)

Storage

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

Suggested protocol for the detection of *Photobacterium damselae* subsp. *piscicida* in fixed tissue sections by immunohistochemistry [Adams, A. and Marin de Mateo, M. (1994)]

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

- 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 Tris buffered saline (TB S) (see buffers).
- 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-*Photobacterium damselae* subspecies *piscicida* 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 TBS.
- Add goat anti-mouse IgG HRP conjugate (1/50 in TBS) to the slides for 30 min
- Wash slides three times with TBS
- To visualise the reaction, incubate the slides for 10 min with DAB solution (see buffers).
- 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 min.
- 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 - the bacterium appears golden brown in colour.

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

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

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

10% (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