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**Anti-Streptococcus iniae
monoclonal antibody**

Product no: P11

Product Information



Product Description

The product is a mixture of two monoclonal antibodies (MAbs) directed against *Streptococcus iniae*. The MAbs have been tested against eight different *Streptococcus iniae* isolates (Type strains and isolates from disease outbreaks) by ELISA and reacted with all the isolates tested.

The specificity of the MAbs has been tested against a range of different bacterial pathogens that infect fish, including *Lactococcus garvae* and *Enterococcus faecalis*, as well as a selection of bacteria found in the aquatic environment (full list available at www.aquaticdiagnosotics.com). Both MAbs were found to cross-react with *Nocardia kampachi* in ELISA. This pathogen results in a very different pathology (granuloma formation) in comparison to *Streptococcus iniae*, enabling positive identification of *iniae* by IHC using Product P11 (see photograph at www.aquatic.diagnostics.com).

Both MAbs are of an IgG2A isotype.



Use of product

The MAbs are recommended for use in immunohistochemistry (IHC). The optimal conditions for use of this product will 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.



Certificate of Analysis

Anti-*Streptococcus iniae* monoclonal antibody

Product no.

Batch no.

Date of expiry

Activity in IHC:

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

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 H₂O₂ (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 % H₂O₂ to 0.5 ml aliquot

NB. DAB is a possible carcinogen

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.

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 *Streptococcus iniae* 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

- .. 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 ($\approx 22^{\circ}\text{C}$) with H_2O_2 in methanol (see buffers).
- .. Wash the slides three times with Tris buffered saline (TBS) (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-*Streptococcus iniae* MAbs 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 the MAbs 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 (see photograph on web site www.aquaticdiagnostics.com).

Buffers

Phosphate buffered saline (PBS)

0.02M Phosphate,	0.15M NaCl	pH adjusted to 7.2 with HCl
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	0.876g/l	
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	2.56g/l	
NaCl	8.77g/l	

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