



*Anti-Tetracapsuloides
bryosalmonae (PKX)
monoclonal antibody*

Product no: P01

Product Information



Product Description

The monoclonal antibody (Mab) against *Tetracapsuloides bryosalmonae* is species-specific and is of an IgG1 isotype. The Mab detects the extrasporogonic stage of the parasite, although occasional *T. bryosalmonae* sporogonic stages may also be recognised.

The specificity of the Mab has been tested against a range of kidney infecting species of Myxozoa, which infect fish.

The Mab tested negative against: *Sphaerospora* sp. in *Salmo trutta*; *Sphaerospora truttae* in *Salmo trutta*; *Sphaerospora* sp. in *Salmo salar*; *Sphaerospora renicola* in *Cyprinus carpio*; *Sphaerospora oncorhynchi* in *Oncorhynchus nerka*; *Sphaerospora elegans* in *Gasterosteus aculaetus*; *Myxidium* sp. in *Anguilla anguilla*; *Sphaerospora* sp. in *Coregonus lavaretus*; *Myxobolus* sp. in *Cottus gobio*; *Myxidium lieberkuehni* in *Esox lucius*; *Chloromyxum* sp., *Myxobolus muelleri*, *Myxobolus* sp., *Sphaerospora* sp. in *Leuciscus leuciscus*; *Sphaerospora* sp. in *Phoxinus phoxinus*; *Myxobolus* sp., *Sphaerospora* sp. in *Rutilus rutilus*; *Chloromyxum* sp. in *Salma trutta*; *Ceratomyxa shasta* in *Oncorhynchus mykiss*; *Myxobolus cerebralis* in *Oncorhynchus mykiss*;

The Mab tested positive against *T. bryosalmonae* in kidneys of: *Salmo trutta*; *Salmo salar*, *Thymallus thymallus*, *Oncorhynchus mykiss*, *Esox lucius* in the UK and for *T. bryosalmonae* from samples obtained from USA, Canada, Denmark, Spain, Ireland, Italy, France, Switzerland.



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 and stored in aliquots. Dilute $1/_{10}$ in PBS before use.



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 *T. bryosalmonae* 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 ($\approx 22^{\circ}\text{C}$) 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-*Tetracapsuloides bryosalmonae* 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 HRP (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 *T. bryosalmonae* parasites appear golden brown in colour.



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

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

3% 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



Relevant References

Adams A, Marin de Mateo, M. (1994) 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.

Marin de Mateo M, Bovo G, Comuzzi M, Adams A (1997) Lectin histochemical studies on Sphaerospora sp. (Myxozoa) from Italian brown trout, *Salmo trutta* L. Journal of Fish Diseases, 20: 51-58.

Marin de Mateo M, McGeorge J, Morris D, Kent ML (1996) Comparative studies of PKX and Sphaerospora spp. from salmonids using lectin and monoclonal antibody staining techniques. Journal of Fish Diseases, 19(1), 55-65.

Morris DJ, Adams, A, Feist SW, McGeorge J, Richards RH (2000) Immunohistochemical and PCR studies of wild fish for *Tetracapsula bryosalmonae*, the causative organism of proliferative kidney disease (PKD) Journal of Fish Diseases 23:129-135.



Certificate of Analysis

Anti-Tetracapsuloides bryosalmonae monoclonal antibody

Product no.

Batch no.

Date of expiry

Activity in IHC: *T. bryosalmonae* parasites appear golden brown in colour when the Mab is used at the working dilution described in the protocol.



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