AQUATIC
Diagnostics Ltd

Anti-Sea bream *(Sparus aurata)* IgM monoclonal antibody labelled with horseradish peroxidase

Product no: C3-HRP

Product Information

Product Description

This monoclonal antibody (Mab) reacts with Sea bream *(Sparus aurata)* immunoglobulin M (IgM) and has been conjugated to horseradish peroxidase. The Mab is of an IgG1 isotype and recognises the heavy chain of the IgM molecule.

Use of product

The Mab is recommended for use in an Enzyme-Linked Immunosorbent Assay (ELISA) to measure antibody levels of antigen-induced IgM. 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.

Vial Contents

The Mab is prepared from bovine-free culture medium and contains no animal-derived stabilisers. Each vial contains sufficient conjugate for five 96-well ELISA plates.

The product should be reconstituted as follows:

- Add 1 ml of phosphate buffered saline (PBS) (see buffers) to the vial to make a stock solution. For use transfer the contents of the vial into 54 ml of antibody buffer.

Storage

Store at -20°C prior to reconstitution. Once reconstituted, the stock solution can be stored at -20°C in 200 µl aliquots and then added to 10.8 ml antibody buffer when required. Repeated freeze/thawing of the product should be avoided.

Protocol

Suggested protocol for the detection of Sea bream IgM by indirect - Enzyme-Linked Immunosorbent Assay (ELISA)

The method outlined here is only a guideline and assay conditions may vary depending on the antigen used to coat the ELISA plate and environmental conditions.

Procedure

The coating procedure depends on the type of antigen used in the screening.

Plates coated with particulate antigens (e.g. bacteria)

- Coat 96-well ELISA plate with 0.05% (w/v) poly-L-lysine in coating buffer, 50 µl well^{-1} for 60 min
- Wash plate with 2 washes of low salt wash buffer
- Resuspend bacteria in PBS (1 x10^{8} bacteria ml^{-1}) and add to the wells of the ELISA plate at 100 µl well^{-1}. Incubate overnight at 4°C or centrifuge plate at x 200 g for 5 min and incubate for 60 min at 22°C
- Add 50 µl well^{-1} 0.05% (v/v) gluteraldehyde, diluted in PBS, to the antigen and incubate for a further 20 min at 22°C

Plates coated with soluble antigen (e.g. fish IgM)

- Coat 96 well ELISA plate with 100 μl well^{-1} antigen [(1-20 μg ml^{-1}) this will need to be optimised by the user] dissolved in coating buffer. Cover and incubate overnight a 4°C.

The remainder of procedure is as follows:

- Wash plate 3 times with low salt wash buffer
- Post-coat plate (to block non-specific binding sites) with either 1% (w/v) bovine serum albumin (BSA) or 3% (w/v) casein (dried milk). Add 250 μl well^{-1} and incubate for 2 h at 22°C
- Wash plate with 3 washes of low salt wash buffer
- Prepare doubling-dilutions of the fish serum in PBS starting with a 1/2 dilution. Also use doubling-dilutions of pre-immune serum or serum from non-vaccinated/non-diseased fish, and PBS as negative controls. Add serum and control dilutions to the wells (100 μl well^{-1}) and incubate for 3 h at 22°C or overnight at 4°C
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add 100 μl well^{-1} of the reconstituted anti-fish Mab-HRP and incubate for 60 min at 22°C
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add 100 μl well^{-1} chromogen in substrate buffer and incubate for 10 min at 22°C
- Stop reaction with 50 μl well^{-1} of stop solution
- Read plate at 450 nm in an ELISA reader. Blank ELISA reader against wells filled with chromogen and stop solution. If an ELISA reader is unavailable compare colour with that of background (i.e. negative control).

NB. WEAR GLOVES WHEN USING CHROMOGEN AND STOP SOLUTION

Buffers

Coating buffer (Carbonate-bicarbonate solution)

Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g

Dissolve in one litre of distilled water. Adjust to pH 9.6.
N.B. prepare fresh coating buffer on each occasion

Phosphate Buffered Saline (PBS)

0.02M Phosphate, 0.15M NaCl

$NaH_2PO_4.2H_2O$	0.876g
$Na_2HPO_4.2H_2O$	2.56g
NaCl	8.77g

Dissolve in one litre of distilled water. Adjust to pH 7.3 with conc. HCl

Wash buffer (x10) (low salt)

Trisma base	24.2 g
NaCl	222.2 g
Merthiolate	1 g
Tween 20	5 ml

Dissolve in one litre of distilled water. Adjust to pH 7.3 with conc. HCl

Wash buffer (x10) (high salt)

Trisma base	24.2 g
NaCl	292.2 g
Merthiolate	1 g
Tween 20	10 ml

Dissolve in one litre of distilled water. Adjust to pH 7.7 with conc. HCl

Antibody buffer

Add 1 g of BSA to 100 ml of PBS (i.e. 1 % BSA solution)

Conjugate buffer

Add 1g of BSA to 100 ml of low salt wash buffer

Substrate buffer (Sodium acetate/ citric acid buffer)

Citric acid	21.0 g
Sodium acetate	8.2 g

Dissolve in one litre of distilled water. Adjust to pH 5.4 with 1 M NaOH

Add 5 μl of H_2O_2 to 15 ml substrate buffer

Substrate

Prepare 3'3'5'5'-Tetramethylbenzidine dihydrochloride (TMB) (42 mM) in 1:2 acetic acid: distilled water. Add 150 μl of this solution to 15 ml substrate buffer

Stop reagent

2M H_2SO_4 in distilled water

Certificate of Analysis

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Product no. C3-HRP

Batch no.

Date of expiry

Absorbance of reconstituted conjugate measured by indirect ELISA:

The reconstituted conjugate gives an absorbance of at 450 nm by ELISA when plates are coated with 10 μg ml^{-1} purified IgM.

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