



Aquatic Diagnostic Ltd.,
Institute of Aquaculture,
University of Stirling,
Stirling, Scotland,
FK9 4LA

telephone: +44 (0)1786 466568
fax: +44 (0)1786 4672133
email: aquaticdiagnostics@stir.ac.uk
<http://www.aquaticdiagnostics.com>



**Anti-Halibut (*Hippoglossus
hippoglossus*) IgM monoclonal
antibody**

Product no: F10

Product Information



Product Description

This monoclonal antibody (Mab) reacts with Halibut (*Hippoglossus hippoglossus*) immunoglobulin M (IgM). The Mab is of an IgG1 isotype and recognises the heavy chain of the molecule.



Use of Product

The Mab is recommended for use in an Enzyme-Linked Immunosorbent Assay (ELISA) for measuring antibody levels of antigen-induced IgM. It can also be used to detect total Halibut (*Hippoglossus hippoglossus*) IgM using an inhibition or sandwich ELISA. 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 three 96-well ELISA plates.

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 32 ml of antibody buffer so that the total volume equals 33 ml



Certificate of Analysis

Anti-Halibut (*Hippoglossus hippoglossus*) IgM monoclonal antibody

Product no.

Batch no.

Date of expiry

Absorbance of reconstituted Mabs by Indirect ELISA

The reconstituted Mab gives an absorbance of _____ at 450nm by ELISA when the plate is coated with 1 µg/ml purified Halibut IgM.

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

Dissolved in one litre of distilled water. Adjusted to pH 5.4 with 1 M NaOH. Add 5 μ l of H₂O₂ to 15 ml substrate buffer

Substrate

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

Stop reagent

2M H₂SO₄ in distilled water



Storage

Store at -20°C 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 Halibut 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 (eg bacteria)

- .. Coat 96-well ELISA plate with 0.05% (w/v) poly-L-lysine in coating buffer, 50 μ l well⁻¹ for 60 min
- .. Wash plate with 2 washes of low salt wash buffer
- .. Resuspend bacteria in PBS (1 x10⁸ bacteria ml⁻¹) and add to the wells of the ELISA plate at 100 μ l 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 μ l well⁻¹ 0.05% (v/v) glutaraldehyde, 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 μ l well⁻¹ antigen [(1-20 μ g 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 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 conjugate (anti-mouse 1gG-HRP diluted 1/1000 in conjugate buffer). 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

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

Phosphate Buffered Saline (PBS)

0.02M Phosphate, 0.15M NaCl
 $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.876g
 $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 2.56g
NaCl 8.77g

Dissolved in one litre of distilled water. Adjusted 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

Dissolved in one litre of distilled water. Adjusted 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

Dissolved in one litre of distilled water. Adjusted to pH 7.7 with conc. HCl