

The logo for AQUATIC Diagnostics Ltd features the word "AQUATIC" in a bold, white, sans-serif font inside a dark blue rectangular box. Below this box, the words "Diagnostics Ltd" are written in a lighter blue, sans-serif font. The background of the entire page is a light blue gradient with decorative wavy lines in various shades of blue.

**AQUATIC**  
Diagnostics Ltd

Anti-Japanese flounder  
(*Paralichthys olivaceus*)  
IgM monoclonal antibody

Product no: F20

Product Information



## Product Description

This monoclonal antibody (Mab) reacts with Japanese flounder (*Paralichthys olivaceus*) immunoglobulin M (IgM). The Mab is of an IgG1 isotype and recognises the light chain of the molecule



## Use of product

The Mab is recommended for use in an Enzyme-Linked Immunosorbent Assay (ELISA) for measuring levels of antigen-induced IgM. 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  $\mu\text{g}$  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 and store as aliquots. Dilute 1/33 in antibody buffer before use.



## Storage

Store at  $-20^{\circ}\text{C}$  prior to reconstitution. For prolonged storage, the Mab solution should be stored at  $-20^{\circ}\text{C}$ . Repeated freeze/thawing of the product should be avoided.



## Protocol

### Suggested protocol for the detection of Japanese Flounder 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,  $50\ \mu\text{l well}^{-1}$  for 60 min
- Wash plate with 2 washes of low salt wash buffer
- Resuspend bacteria in PBS ( $1 \times 10^8$  bacteria  $\text{ml}^{-1}$ ) and add to the wells of the ELISA plate at  $100\ \mu\text{l well}^{-1}$ . Incubate overnight at  $4^{\circ}\text{C}$  or centrifuge plate at  $\times 200\ \text{g}$  for 5 min and incubate for 60 min at  $22^{\circ}\text{C}$
- Add  $50\ \mu\text{l well}^{-1}$  0.05% (v/v) glutaraldehyde, diluted in PBS, to the antigen and incubate for a further 20 min at  $22^{\circ}\text{C}$

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

- Coat 96 well ELISA plate with  $100 \mu\text{l well}^{-1}$  antigen [(1-20  $\mu\text{g ml}^{-1}$ ) this will need to be optimised by the user] dissolved in coating buffer. Cover and incubate overnight at  $4^{\circ}\text{C}$ .

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  $250 \mu\text{l well}^{-1}$  and incubate for 2 h at  $22^{\circ}\text{C}$
- 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 ( $100 \mu\text{l well}^{-1}$ ) and incubate for 3 h at  $22^{\circ}\text{C}$  or overnight at  $4^{\circ}\text{C}$
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add  $100 \mu\text{l well}^{-1}$  of the reconstituted anti-fish Mab-HRP and incubate for 60 min at  $22^{\circ}\text{C}$
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add  $100 \mu\text{l well}^{-1}$  conjugate (anti-mouse 1gG-HRP diluted  $1/1000$  in conjugate buffer). Incubate for 60 min at  $22^{\circ}\text{C}$
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add  $100 \mu\text{l well}^{-1}$  chromogen in substrate buffer and incubate for 10 min at  $22^{\circ}\text{C}$
- Stop reaction with  $50 \mu\text{l well}^{-1}$  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)

$\text{Na}_2\text{CO}_3$  1.59 g

$\text{NaHCO}_3$  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

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  0.876g

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  2.56g

NaCl 8.77g

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  $\mu$ l of H<sub>2</sub>O<sub>2</sub> 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  $\mu$ l of this solution to 15 ml substrate buffer

### Stop reagent

2M H<sub>2</sub>SO<sub>4</sub> in distilled water



## Certificate of Analysis

Anti-Japanese flounder (*Paralichthys olivaceus*) monoclonal antibody

Product no. F20

Batch no.

Date of expiry

### **Absorbance of reconstituted Mabs by indirect ELISA:**

The reconstituted conjugate gives an absorbance of  
at 450 nm by ELISA when plate is coated with  $10 \mu\text{g ml}^{-1}$  purified IgM.



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