AQUATIC Diagnostics Ltd

Anti-Zebrafish *(Danio rerio)* IgM monoclonal antibody

Product no: F25

Product Information

Product Description

This monoclonal antibody (Mab) reacts with Zebrafish *(Danio rerio)* immunoglobulin M (IgM). The Mab is of an IgG2b 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. 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 200μg 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 1ml 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°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.

Protocol

Suggested protocol for the detection of Zebrafish 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, 50μl well^{-1} for 60min
- 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 100μl well^{-1}. Incubate overnight at 4°C or centrifuge plate at x 200 g for 5min and incubate for 60min at 22°C
- Add 50μl well^{-1} 0.05% (v/v) gluteraldehyde, diluted in PBS, to the antigen and incubate for a further 20min at 22°C

Plates coated with soluble antigen

- Coat 96 well ELISA plate with 100μl well^{-1} antigen [(1-20μg ml^{-1}) this will need to be optimised by the user] dissolved in coating buffer. Cover and incubate overnight at 4^{o}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 250μl well^{-1} and incubate for 2h at 22^{o}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 3h at 22^{o}C or overnight at 4^{o}C
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add 100μl well^{-1} of the reconstituted anti-fish Mab and incubate for 60 min at 22^{o}C
- Wash plate with 5 washes of high salt wash buffer, incubating for 5 min on last wash
- Add 100μl well^{-1} conjugate (anti-mouse IgG-HRP diluted 1/1000 in conjugate buffer). Incubate for 60 min at 22^{o}C
- Wash plate with 5 washes of high salt wash buffer, incubating for 5min on last wash
- Add 100μl well^{-1} chromogen in substrate buffer containing $H_2O_2$and incubate for 10min at 22^{o}C
- Stop reaction with 50μl 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	1.59g
$NaHCO_3$	2.93g

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

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

Wash buffer (x10) (high salt)

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

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

Antibody buffer

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

Conjugate buffer

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

Substrate buffer (Sodium acetate/ citric acid buffer)

Citric acid	21.0g
Sodium acetate	8.2g

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

Add 5μl of H_2O_2 (substrate) to 15ml substrate buffer

Chromogen

Prepare 3'3'5'5'-Tetramethylbenzidine dihydrochloride (TMB) (42 mM) in 1:2 acetic acid: distilled water. Add 150μl of this solution to 15ml substrate buffer containing H_2O_2

Stop Solution

2M H_2SO_4 in distilled water

Certificate of Analysis

Anti-Zebrafish *(Danio rerio)* **IgM monoclonal antibody**

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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 10 $\mu g ml^{-1}$ purified Zebrafish IgM

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