



## User's Manual

# Japanese medaka vitellogenin ELISA kit

REF

DEIABL459



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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

The assay has been developed for quantification of Vtg in plasma, liver homogenates and whole body homogenate (wbh) samples, but may also be used with other sample types.

### General Description

Detection of the egg yolk precursor vitellogenin (Vtg) in blood and tissue samples of juvenile and male fish is a simple and sensitive biomarker for endocrine disrupting chemicals (EDCs) with oestrogenic effects. Measurement of Vtg has become an accepted routine screening test for oestrogenic and anti-androgenic effects of EDCs in fish. This Enzyme- Linked Immunosorbent Assay (ELISA) can readily be combined with standard fish toxicology tests developed under the framework of governmental organizations, e.g. the OECD and the US EPA.

### Principles of Testing

This ELISA utilises specific binding between antibodies and vitellogenin (Vtg) to quantify Vtg in samples from medaka. The wells of microplates have been pre-coated with a specific Capture antibody that binds to Vtg in standard and sample added to the wells. A different Vtg-specific Detecting antibody, labelled with the enzyme horseradish peroxidase (HRP), is added to create a sandwich of Vtg and antibodies. The enzyme activity is determined by adding a substrate that gives a coloured product, and the colour intensity is directly proportional to the amount of Vtg present.

### Reagents And Materials Provided

- A) 96 well microplates, pre-coated
- B) Plate sealers
- C) PBS/Tween tablets
- D) Dilution buffer, concentrated 5x
- E) Detecting antibody, concentrated 500x
- F) TMB substrate
- G) Medaka Vtg standard

### Materials Required But Not Supplied

- 0.3M H<sub>2</sub>SO<sub>4</sub> (stop solution)
- Microplate reader (wavelength 450 nm)
- Pipettes with disposable tips (5-1000 µl)
- Multi-channel pipette and reagent reservoir. Alternatively, a stepper pipette with disposable tips (100 µl) can

be used.

- Test tubes (1-50 ml)
- Microplate washing device (an automatic or manual plate washer is recommended, but a squeeze bottle or a multichannel/stepper pipette can also be used)
- Vortexer
- Crushed ice

## Storage

Store the kit at 2-8°C upon arrival. Do not freeze. See expiry date on the kit box for stability of the kit. Unused pre-coated microplates should be stored airtight with enclosed desiccant at 2-8°C.