EnBio Medaka Vitellogenin ELISA system

Code EBT-MEV-ELISA-EX
96 wells

STORAGE :
Store at -15~-30℃

EXPIRY :
The expiry date is started on the package and will be at least 4 weeks from the date of dispatch.

Warning
For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

This system does not contain medaka vitellogenin standard. When the standard is needed, it is supplied as 'Vitellogenin, Medaka Standard Set'.

INTRODUCTION

Vitellogenin is an egg yolk protein precursor and is little detected in the body of male fish. The concentration of the precursor is elevated drastically in plasma when male fish is exposed to estrogenic chemicals. In such phenomenon, vitellogenin has been proposed as an ideal biomarker for the detection of the estrogenic effects of endocrine disrupting chemicals (EDCs).

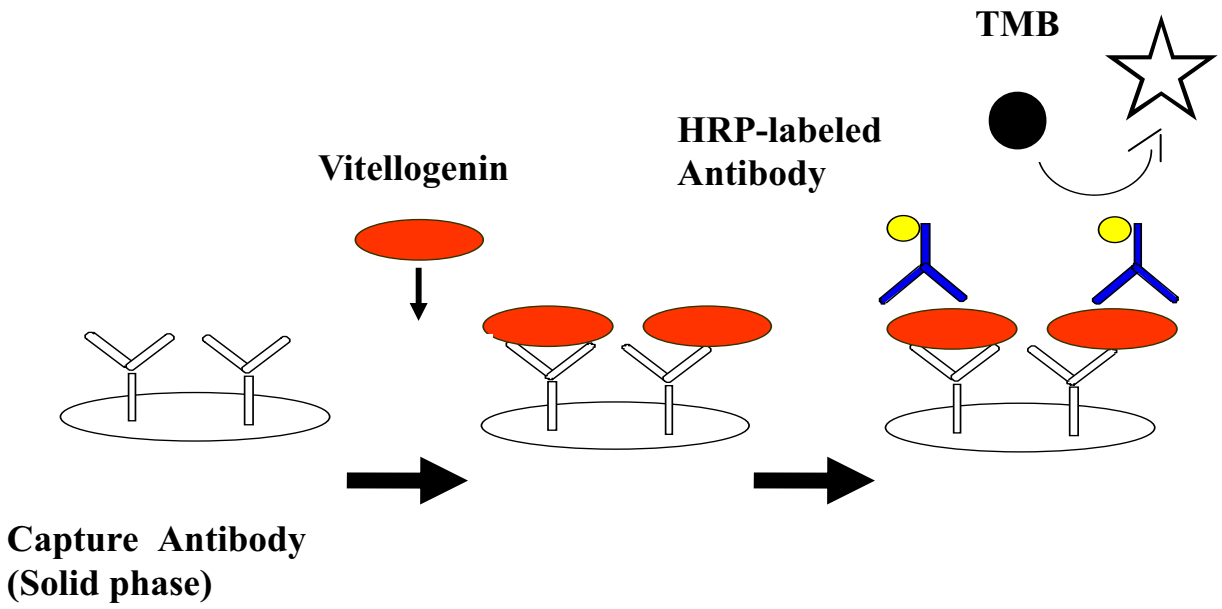
EnBio Medaka vitellogenin ELISA system is based on monoclonal antibodies against Medaka vitellogenin and highly sensitive due to use the specific antibodies. Each pack contains sufficient material for the vitellogenin assay and 40 samples can be measured in duplicate.

1. High sensitivity
2. Total incubation time of 3 hours
3. Pre-coated plate
4. Specific for Medaka vitellogenin
5. Assay Range, 2-100 ng/ml
6. Small sample size, 50µl per well
7. Non-isotope assay system

PRINCIPLE

The EnBio Medaka Vitellogenin ELISA is using monoclonal antibodies specific to Medaka vitellogenin. The assay is based on a solid phase ELISA 'sandwich format'. Standards and samples are incubated in microtitre wells precoated with an anti-vitellogenin monoclonal antibody. Any vitellogenin present will be bound to the wells, other components of the sample being removed by washing and aspiration. Bound analyte is then detected using a HRP labeled anti-vitellogenin monoclonal antibody.

HRP activity is determined by the addition of TMB substrate solution. The reaction is stopped by addition of an acid solution and the resultant color read at 450nm in a microtitre plate spectrophotometer. The concentration of vitellogenin in a sample is determined by interpolation from the standard curve.



ELISA SYSTEM CONTENTS

1.Antibody coated microtiter plate (6 strips of 16 wells), 1 plate

2.Assay buffer, 50mL, 1 vial

3.Wash buffer concentrate, 40mL, 1 vial

4.Antibody-HRP concentrate, 0.07mL, 1 vial

5.Antibody-HRP dilution buffer, 7mL, 1 vial

6.TMB substrate, 1 vial

7.Stop solution, 7mL, 1 vial

8.Plate cover, 2 sheets

SAFETY WARNINGS AND PRECAUTION

Warning: For research use only. Not for clinical diagnostic use. Do not use internally or externally in humans or animals. Avoid contact with skin or eyes.

We recommend that this product and components are handled only by those persons who have been trained in laboratory techniques. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves.

Warning: Contains sodium azide as preservative.
Dispose of waste by flushing with copious amount of water to avoid the build up of explosive metallic azides in copper and lead plumbing. The total azide present in each kit is 11 mg.

GENERAL NOTES

1. Read the complete procedure before starting the assay.
2. Allow all reagents to reach room temperature prior to performing the assays.
3. Avoid handling the tops and bottoms of the wells both before and after filling.
4. Standards and samples should be assayed in duplicate.
5. Run a separate standard curve for each assay.
6. The total dispensing time for each plate should not exceed 20 minutes.
7. Use only coated wells from the same reagents batch for each assay. Also do not mix reagents from different kit lots.
8. New pipette tips should be used for each standard and sample.
9. It is important that the wells are washed thoroughly and uniformly. If using automatic washer check operation of head before starting. If washing by hand ensure that all wells are completely filled at each wash.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

The following materials and equipment are required:

For ELISA assay

1. Pipettes or pipetting equipment with disposable tips (1-20µl, 20-200µl and 100-1, 000µl).
2. Multiple pitette (8 channels)
3. Disposable polypropylene test tubes
4. 400ml measuring cylinder
5. Distilled or deionized water
6. Plate reader capable of reading at 450nm
7. Plate shaker

For plasma sample preparation

1. Disposable 1.5ml plastic tubes
2. Ethyl 3-aminobenzoate-10 methnesulfonic acid salt (for anesthesia; Working concentration is 1mg/1ml in distilled water)
3. Disposable laboratory dish (ϕ=60mm)
4. Scalpel
5. Disposable calibrated capillary tube (5µl)
6. Refrigerated micro-centrifuge
7. Forceps
8. Micropipette (250µl)

SAMPLE PREPARATION

The followings are procedure of sample preparation.

1. Dispense 250 µl of assay buffer into a 1.5 ml plastic tube and stand it on ice.
2. Put a Medaka under anesthesia in a 60mm laboratory dish.
3. Hold anesthetized Medaka in your left hand and cut a ventral portion of the fin with a scalpel on the cover of a 60mm laboratory dish.
4. Immediately pick a blood up into a capillary tube.
5. The blood is transferred to the 1.5 ml plastic tube by breathing into the capillary tube.
6. Centrifuge at 8, 000 rpm for 10 min at 4℃.
7. Supernatant is used as ELISA sample.

Plasma samples should be stored at 2-8℃ and assayed within 24 hours. If storage for long period is needed, the samples should be frozen at -70℃. Avoid freezing and thawing .

ELISA PROCEDURE

Reagent preparation

Note: All reagents must be allowed to equilibrate to room temperature before use. This is particularly important for the enzyme substrate (TMB).

1. Either distilled or deionized water may be used for reagent preparation.
2. The microtitre plate, substrate and stop reagent are supplied ready to use when equilibrated to room temperature.
3. Assay buffer should be mixed well prior to use.

Wash buffer

Transfer the contents of the bottle containing wash buffer concentrate (40 ml) to a 400 ml measuring cylinder. Adjust the final volume to 400 ml with deionized water and mix thoroughly. Store at 2-8 ℃ for up to one month.

Antibody -HRP conjugate

Antibody-HRP concentrate is diluted immediately prior to use. Calculate and prepare the working volume of the reagent. (When whole plate is used at the same time, dilute 60 µl of antibody-HRP conjugate into 6ml of antibody-HRP dilution buffer in a polypropylene tube and mix well.) Remaining solution can be stored at 2-8 ℃ for up to the expiry date.

Working standards

Medaka vitellogenin standard and standard diluent are supplied as ‘Vitellogenin, Medaka Standard Set’. For the details, see the attached sheet of the set.

Assay protocol

Decide the number of strips you wish to run, leaving the strips to be used in the frame. Remove the unnecessary strips and store them with plate cover in the foil pouch at 2-8℃, making sure the foil pouch is sealed tightly. After running the assay, retain the plate frame for the second partial plate. The strips can be stored at 2-8℃ for up to the expiry date.

When adding the TMB substrate reagent, pour out from the bottle only the amount needed to run the plate. Care must be taken to ensure that the remaining TMB substrate reagent is not contaminated. If the substrate reagent is bright blue prior to use, it has been contaminated. DO NOT USE.

1. Prepare the wash buffer and antibody-HRP conjugate as described in the previous section.

Note: Working standard is supplied as 'Vitellogenin, Medaka Standard Set'. When the set is used as the assay standard, prepare the working standards as described in the attached sheet of the set.

2. Set up the microtitre plate with sufficient wells for running all blanks (zero standard), standards and samples as required. We recommend that all standards and samples are assayed duplicate.
3. Wash all wells 3 times with wash buffer, ensuring that the wells are completely filled and emptied at each wash. After third wash, blot the plate by tapping briskly on tissue paper.
4. Pipette 50 µl of assay buffer into the wells as a zero standard.
5. Pipette 50 µl of each standard into the appropriate wells.
6. Pipette 50 µl of each sample into the appropriate wells.
7. Cover the plate with the plate cover sheet and incubate at room temperature (20-28℃) for 1 hour with shaking on a plate shaker moderately.
8. Aspirate and wash all wells 3 times as step 4.
9. Pipette 50 µl of diluted antibody-HRP conjugate as step 1 into each well.

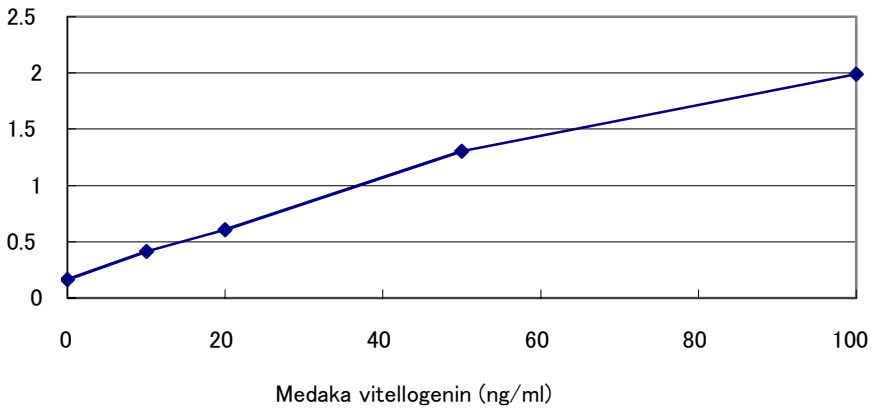
10. Replace the plate cover sheet and incubate at room temperature (20-28℃) for 1 hour with shaking on a plate shaker moderately.
11. Aspirate and wash all wells 3 times as step 3.
12. Pipette 50 µl of room temperature equilibrated substrate TMB into all wells.
13. Incubate the plate at room temperature (20-28℃) for 20 minutes. Do not shake the plate in this incubation step.
14. Add 50 µl of stop solution to each well.
15. Determine the optical density of each well within 10 minutes using a spectrophotometer set to 450nm.

DATA PROCESSING

Calculation of results

Typical assay data are shown in table 1. Average the duplicate readings for each standard, control and sample.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve (figure 1). A standard curve should be generated for each assay.



Typical assay data

Table 1. Typical assay data

	Optical density	Zero standard subtracted
Zero standard	0.162	—
10ng/ml standard	0.412	0.250
20ng/ml standard	0.606	0.444
50ng/ml standard	1.303	1.141
100ng/ml standard	1.990	1.828

ADDITIONAL INFORMATION

Specificity

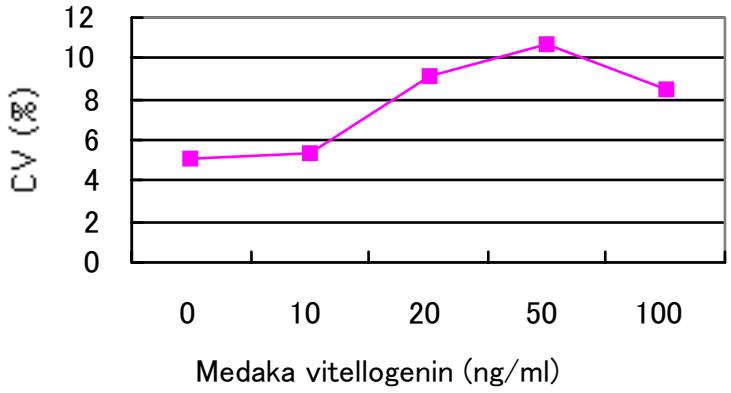
Antibodies in this system react to vitellogenin in Medaka specifically. No cross-reactivity was found against other components in Medaka plasma.

Sensitivity

The sensitivity, defined as the concentration on the standard curve equivalent to 2 standard deviation above zero standard (n=10), was determined to be 2ng/ml.

Precision

A within-assay precision profile was generated by assaying 6 replicates of each of the standards. The results are shown in figure 2.



Intra- and inter-assay variation

An intra-assay variation was generated by assaying 10 replicates of each control plasma. An inter-assay variation was generated by assaying 6 replicates of each control plasma. The results are shown in table 2 and table 3.

Table 2. Intra-assay

Sample	Mean (ng/mL)	SD	CV(%)	n
High	79.3	4.66	5.6	10
Med	38.3	2.37	6.2	10
Low	18.7	1.44	7.7	10

Table 3. Inter-assay

Sample	Mean (ng/mL)	SD	CV(%)	n
High	75.1	1.66	2.2	6
Med	39.1	1.88	4.8	6
Low	17.4	1.49	8.5	6

Recovery

Recovery was determined by plasma samples into an equal volume of control plasma. Recovery for all samples was within the range of 92-111% .

Linearity of dilution

Linearity of dilution was determined by diluting plasma samples x20, x40, x80 and x160 fold. Results from all samples were in the range of 84-114% of expected value.

Troubleshooting guide

1. Low optical densities

1.1) Check the reader wavelength at 450nm.

1.2) Check reagents have been correctly reconstituted.

1.3) Check reagents have been stored under the recommended conditions.

1.4) Check incubation time and temperatures.

1.5) Ensure all reagents have been equilibrated to room temperature before use.

1.6) Ensure that the plate is read within 30 minutes of adding the stop reagent.

2. High optical density

3.1) Check point 1.2.

3.2) Check point 1.4.

3.3) Ensure the every wash step in the assay procedure is carried out completely.

4. Poor replication or precision

4.1) Ensure automatic washers are working correctly, or that each well is completely filled and emptied at every wash step when hand washing.

4.2) Check pipette calibration

4.3) Ensure troughs used with multichannel pipettes are dedicated to individual components.

4.4) Ensure that plates have been carefully placed into the shaking incubator and the plate reader, to avoid splashing and resultant cross contamination of the wells.

4.5) Check standard dilution procedure.

Distributor

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