Vitellogenin (zebrafish) ELISA Kit

Cat. No.:DEIA5068
Pkg.Size:96T

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 (arukwe & goksøyr, 2003; sumpter & jobling, 1995). 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.

Principle Of The Test
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: 1
B) Plate sealers: 2
C) Phosphate Buffered Saline (Pbs) Tablets: 2
D) PBS/TWEEN Tablets: 1
E) Bovine Serum Albumin (Bsa): 2 g
F) Detecting Antibody, Concentrated 350X: 1 vial
G) Secondary Antibody, Concentrated 2000x: 1 vial
H) Opd-Peroxidase Substrate, Tablet Sets: 1 set
I) Zebrafish Vtg Standard *: 1 vial
Purified, Lyophilized Vtg From Zebrafish

Materials Required But Not Supplied
In addition to the reagents supplied with the kit, the following reagents and equipment are required and/or recommended to perform the assay:
1. 2M H2SO4 (stop solution)
2. Microtiter plate reader (wavelength 492 nm)
3. Pipettes with disposable plastic tips (5-1000 µl)
4. Multi-channel or stepper pipette with plastic tips (50 and 100 µl)
5. Test tubes (1-50 ml)
6. Microtiter plate washing device (a manual or automatic plate)
7. Washer is recommended, but a squeeze bottle or a multichannel/stepper pipette can also be used
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 precoated microplates should be stored airtight with enclosed desiccant at 2-8°C.

Reagent Preparation

1. Dilution buffer: PBS (Phosphate buffered saline, pH 7.3), 1% BSA: Dissolve one buffer tablet (plastic bag C) and 1.0 g BSA (vial E) per 100 ml Distilled water. Store at 2-8°C (stable for 2-3 days).
2. Washing buffer (PBS, 0.05% tween-20): 1-plate kit: dissolve one small buffer tablet (bag D) in 500 ml distilled water. 5-plate kit: dissolve one large buffer tablet (bag D) in 1000 ml distilled water. Store at 2-8°C (stable for at least one month).
3. Substrate solution (prepare just prior to use): Dissolve one urea hydrogen peroxide tablet in 20 ml distilled water (dissolves slowly, 10-15 minutes with gentle shaking), then add one OPD tablet and let it dissolve. The substrate solution should be used within 30 minutes. Warning: OPD (o-phenylenediamine) is toxic and may cause cancer. Avoid contact. Use gloves and suitable protective clothing when handling tablets and substrate solution.

Assay Steps

Preparing dilutions of standard and samples:
Please note: VTG is an unstable molecule, and all standard and sample dilutions should be prepared and kept on ice. Frozen samples should be thawed on ice.
1. Dilution of the VTG standard:
Dissolve the content of one vial of zebrafish VTG standard (vial I) in 1.0 ml cold Dilution buffer. Please note: release the vacuum in the vial carefully. Do not remove the grey Rubber stopper completely, but add the buffer through the opening at the side. Recap the vial and mix carefully by tipping and vortexing. Avoid foaming. Ensure that all material in the vial is dissolved. Calculate the concentration of VTG in this stock solution based on the VTG amount specified on the vial (µg per vial). Prepare the first dilution step for the standard curve by diluting 50 µL of the stock solution in an appropriate volume of cold dilution buffer to give a solution of 125 ng zebrafish VTG/ml.
Example: A vial containing 10 µg VTG dissolved in 1.0 ml cold dilution buffer gives a solution of 10 µg zebrafish VTG/ml. Prepare the first dilution step for the standard curve (125 ng/ml) by adding 50 µL of the 10 µg/ml solution into 3950 µL dilution buffer.
Prepare a two-fold serial dilution in dilution buffer (e.g. 500 µL zebrafish VTG dilution + 500 µL buffer for each standard curve run in the assay). The standard series should include 11 dilution steps, ending with a concentration of 0.12 ng Zebrafish VTG/ml. Keep the dilutions on ice until use.
2. Dilution of whole body homogenate samples:
Given the wide range of VTG levels found in experimental studies, we recommend preparing at least three different dilutions of each sample in order to hit the linear part of the standard curve. Mix the homogenate samples well before preparing the dilutions.
We recommend preparing a 1:500 dilution (add 5 µL sample to 2495 µL cold dilution buffer), a 1:30 000 dilution (add 10 µL of the 1:500 dilution to 590 µL cold dilution buffer) and a 1:1 800 000 dilution (add 10 µL of the 1:30 000 dilution to 590 µL cold dilution buffer). Keep the dilutions on ice until use.

Incubation with standard and diluted samples:
Please note: when more than one plate is run in the assay, complete the addition of both standard and sample dilutions on one plate before proceeding to the next plate.
3. Add 100 µL dilution buffer to each of the two NSB wells.
4. Add in duplicate 100 µL of each zebrafish VTG standard dilution.
5. Add in duplicate 100 µL of each sample dilution.
6. Seal the plates and incubate at room temperature (20-25°C) for 1 hour.

Incubation with detecting antibody:
7. Dilute the detecting antibody (vial f) 1:350 by adding 31 μl to 11 ml dilution buffer for each plate run in the assay.
8. Wash the plates three times with 200 μl washing buffer per well.
9. Add 100 μl of the diluted detecting antibody to all wells.
10. Seal the plates and incubate at room temperature (20-25°C) for 1 hour.

Incubation with secondary antibody:
11. Dilute the secondary antibody (vial g) 1:2000 by adding 6 μl to 12 ml dilution buffer for each plate run in the assay.
12. Wash the plates three times with 200 μl washing buffer per well.
13. Add 100 μl of the diluted secondary antibody to all wells.
14. Seal the plates and incubate at room temperature (20-25°C) for 1 hour.

Development

Please Note: the substrate solution should be prepared just before proceeding to the next step.
15. Wash the plates five times with 200 μl washing buffer per well.
16. Add 100 μl substrate solution to all wells.
17. Incubate: in the dark (cover the plates with e.g. aluminium foil) at room temperature (20-25°C) for 30 minutes.
18. Stop the reaction by adding 50 μl 2m h2so4 to all wells.
19. After five minutes, read the absorbance at 492 nm with a microtiter plate reader.

Calculation

Subtraction of nsb absorbance values:
On each plate, calculate the mean of the absorbance values of the two nsb wells and subtract this value from the absorbance values of all other wells on the same plate. This gives the nsb-corrected absorbance values for standard and sample dilutions.

Typical Standard Curve

Preparation of the standard curve:
1. calculate the mean of the nsb-corrected absorbance values for each set of Standard duplicates.
2. Plot absorbance values against the vtg concentration. Perform a regression Analysis, using for example log-log, linear or 4-parameter transformation of the data. Please note: a 4-parameter transformation will often give a wide working range, but is often best suited for standard curves with defined plateaus (as in competitive assays). Care should be taken when employing
such a model in this assay. The model will be sensitive to the exclusion of data points, and the upper and lower ends of the curve should be used with care.

3. To determine the working range of the standard curve, omit data points using the following guidelines:
   • The correlation coefficient (r²) should be higher than 0.990 (a perfect regression has an r² value of 1.0). If the r² value is lower than 0.990, exclude points that deviate from the line (usually at the ends) until it is above 0.990.
   • Data points that clearly deviate from the regression line should not be included, even if the r² value is above 0.990.
   • Data points with nsb-corrected absorbance values lower than 0.010 should not be included in the working range.

Calculation of vtg concentration in the samples:
4. Calculate the mean of the nsp-corrected absorbance values for each set of sample duplicates.
5. Calculate the vtg concentration in the diluted sample using the equation for the adjusted standard curve determined above (pt 2-3). Use the following guidelines when determining the vtg concentration in the samples:
   Please note: only sample dilutions with absorbance values that fall within the standard curve working range should be used (see example below). If all dilutions of a sample give absorbance values outside the working range, the sample should be reassayed at different dilutions.
6. Multiply the vtg concentration in the diluted sample with the dilution factor to get the vtg concentration in the original sample.
   Please note: if more than one dilution of a sample fall within the standard curve working range, the mean vtg concentration should be calculated. If the different dilutions yield contrasting results, care should be taken to determine which of the dilutions is the most reliable one. Samples having absorbance values close to the ends/plateaus of the standard curve should be used with care, as these parts of the standard curve are less reliable. Alternatively, the sample should be reassayed with more dilutions.

Reproducibility
Intra-Assay Precision (CV%): 3.6-6.8
Inter-Assay Precision (CV%): 13-21

Precautions
For research use only. Not for human use or drug use, not for clinical diagnostic use. These reagents contain sodium azide as preservative. Do not use internally or externally in humans or animals. As all chemicals should be considered potentially hazardous, it is advisable to wear suitable protective clothing during handling of this kit. Avoid contact with skin and eyes.

REFERENCES
• Brion f., nilsen b.m., eidem j.k., goksøyr a. & porcher j.m. (2002). Development and validation of an enzyme-linked immunosorbent assay to measure vitellogenin in the zebrafish (danio rerio). Environ. Toxicol. Chem. 28, 1699-1708.