

Detection of the egg yolk protein vitellogenin (Vtg) in juvenile and male fish is a simple and sensitive biomarker for endocrine disrupting chemicals (EDCs) with estrogenic effects in fish. Measuring Vtg has become accepted as a routine screening test for estrogenic effects of EDCs in fish. This quantitative Vtg Enzyme-Linked Immunosorbent Assay (ELISA) can easily be combined with standard fish tests according to OECD Guidelines for Testing of Chemicals.

The quantitative Fathead minnow Vtg ELISA Kit is developed for a standard OECD test species used in many ecotoxicological testing laboratories throughout the world, the fathead minnow (*Pimephales promelas*). The assay can be used for analysis of both blood plasma and whole body homogenate samples).



FATHEAD MINNOW (PHOTO: C. TYLER)

The analysis is based on a homologous sandwich assay utilizing specific binding between antibodies and Vtg. Microtiter plates are **pre-coated** with a Vtg-specific capture antibody, which binds Vtg in samples added to the wells. Unbound components are washed out, and a second Vtg-specific, enzyme-labelled antibody (detecting antibody) is added, creating a sandwich of antibodies and Vtg. Unbound detecting antibody is washed out and the

enzyme activity is determined by adding a substrate which gives a coloured product. The enzyme activity (colour intensity) is directly proportional to the concentration of Vtg in the sample, and the assay is calibrated using purified Vtg from fathead minnow as standard.

Validation data:

SINGLE-LAB VALIDATION

Selectivity (matrix effect)

No response at minimum dilution = 1:50 (plasma), 1:100 (WBH)

Calibration and working range:

Linear range: 0.1-25 ng/ml; 250-fold
Within-day repeatability precision (%CV): 6.0 %
Between-day repeatability precision (%CV): 7.7 %

Accuracy (Recovery)

75% (samples frozen and thawed once)
79-106% (freshly spiked samples)

Limit of Detection (LoD), Limit of Quantification (LoQ)

LoD: 0.02 ng/ml (plasma), 0.04 ng/ml (WBH)
LoQ: 0.09 ng/ml (plasma), 0.11 ng/ml (WBH)

Sample LoQ (= LOQ x necessary minimum matrix dilution)

Plasma: 4.68 ng/ml (1:50)
WBH: 11.35 ng/ml (1:100)

Precision (intra- and inter-assay variation)

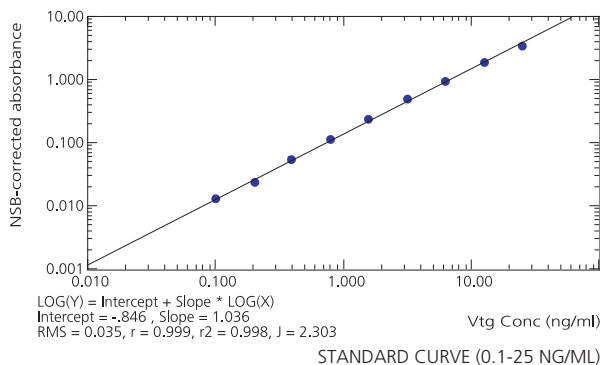
Within-day repeatability precision (%CV): 4.5 %
Between-day repeatability precision (%CV): 9.9 %

Ruggedness

Elevated buffer and incubation temperature have strongest effects on quantification of Vtg.

Comparison with existing methods:

Biosense Carp Vtg ELISA kit: $R^2 > 0.99$
Competitor FHM Vtg ELISA kit: $R^2 > 0.99$



INTER-LAB VALIDATION

Precision (inter-assay and inter-lab variation):

Between-day Repeatability Precision (%CV): 16.4%
Between-lab Reproducibility Precision (%CV): 18.6%

Accuracy (Recovery):

69.4 % (samples frozen and thawed once)

The Fathead minnow Vtg ELISA Kit comes in two sizes, with 96 and 480 wells (1 and 5 plates). The kit contains pre-coated microtiter plates, fathead minnow Vtg standard, antibodies and reagents necessary for running the assay, as well as an instructive protocol.