



TECO[®] Vitellogenin ELISA System in Fish

For Research Use Only

For use with **blood, homogenate, epidermal mucus or cell culture samples**

TECO[®] Vitellogenin ELISA assays:

- ✓ Developed and produced under ISO 13485 und ISO 9001 standards;
- ✓ Minimum shelf life of 12 months from production;
- ✓ Test kits are designed for 2 assay runs per kit;
- ✓ Test kits contain at least 2 controls;
- ✓ Sample preparation and assay take place at room temperature;
- ✓ Buffer system allows for parallel measurement of protein concentration in samples;
- ✓ Assays validated for use with the following sample types: blood, homogenate (head, tail, liver, brain), epidermal mucus, gill swab & cell culture

The 1st validated test application epidermal mucus:

- Allows for *repeated* non-invasive, non-destructive sampling
- Follows the “Three R’s principle” (Replacement, Reduction, Refinement)
- Sampling method of choice for outdoor field projects

○ applications for TECO[®] Vitellogenin ELISAs

- For protein testing in more than 35 different fish species (including marine)
- Vitellogenin determination in juvenile, male or female fish, or cell culture
- Chemical testing according to OECD Guidelines
- Environmental monitoring and ecotoxicology assessment

Introduction

Vitellogenin in fish is an estrogen induced yolk precursor protein mainly synthesized in the liver to be deposited in maturing oocytes, where it is split into the yolk proteins lipovitellin 1, lipovitellin 2 and phosvitin. These yolk proteins serve as nourishment for developing embryos. Due to its estrogen-dependent synthesis and its significance in the nutrition of the offspring, vitellogenin is considered a typical “female protein”.

Because male and juvenile fish produce few estrogens, their vitellogenin levels are typically quite low. Non-physiological induction of vitellogenin in such fish is thought to indicate an estrogen mediated endocrine disruption. Therefore, vitellogenin determination is one of the core endpoints in screening and testing for endocrine disrupting chemicals standardized in the OECD Guidelines for the testing of chemicals for estrogenic activity (1,2,3).

Vitellogenin is a precursor protein that is cleaved into yolk proteins (see above) and therefore it is a very unstable protein. For this reason samples containing vitellogenin have to be frozen immediately after collection.

Normally vitellogenin is measured in blood samples or whole body homogenate (WBH) - both sample types require invasive and destructive treatment of the fish. Blood is difficult to collect, particularly in very small fish where the animals must survive sampling (5).

While the processing of blood samples is not a problem in the laboratory, the centrifugation and freezing of blood samples are difficult to do in ecotoxicological studies in the field.

Recently, different studies have shown that vitellogenin can be measured in the epidermal mucus of fish (4,6). The vitellogenin concentrations in mucus are significantly lower compared to blood and homogenates. Therefore suitable methods for mucus collection and vitellogenin determination are required.

The TECO Vitellogenin System is unique in that it includes a validated mucus collection set for gentle and effective mucus sampling while also being adapted for high-sensitivity laboratory assays.

The TECO Vitellogenin System in epidermal mucus offers following advantages:

- Simple and highly standardized sampling technique and sample preparation;
- Non-invasive and non-destructive sampling procedure;
- Minimum 2 samples per fish at one time;
- Several subsequent samplings possible;
- Defined matrix without protease contamination caused by non-target tissues or lymphatic fluid;
- Optimal procedure to obtain vitellogenin samples outdoors
 - a) Mucus Collection Set for fast and simple sampling
(all components required to collect the samples are included);
 - b) Mucus samples can be frozen immediately after collection on dry ice.

TECO® Mucus Collection Set (TE1034)

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The Mucus Collection Set includes validated swabs, sample tubes, a sample tube rack, kit instructions and a specially designed extraction buffer for use in TECO® Vitellogenin ELISAs. The Mucus Collection Set is a validated tool for repeatable non-invasive/non-destructive sampling of epidermal mucus for vitellogenin determination. Patent submitted (PCT/DE2014/100161)

Procedure

1. Collect the epidermal mucus by swabbing the fish from head to tail.



2. Transfer the swab tip into the vial by breaking the swab at designed breaking point.



3. Store the mucus-loaded swab tip at -20°C until the ELISA test is performed.

Additional test applications for epidermal mucus using the TECO® Mucus Collection Set

- ✓ **Cortisol:** Stress marker (TECO®Mucus-Cortisol EIA, TE1052, for Research Use Only)
- ✓ **Hyaluronic Acid:** Liver toxicity* (TECO®Hyaluronic Acid ELISA, TE 1018-2, for Research Use Only)

*validation studies ongoing

TECO® Vitellogenin ELISA

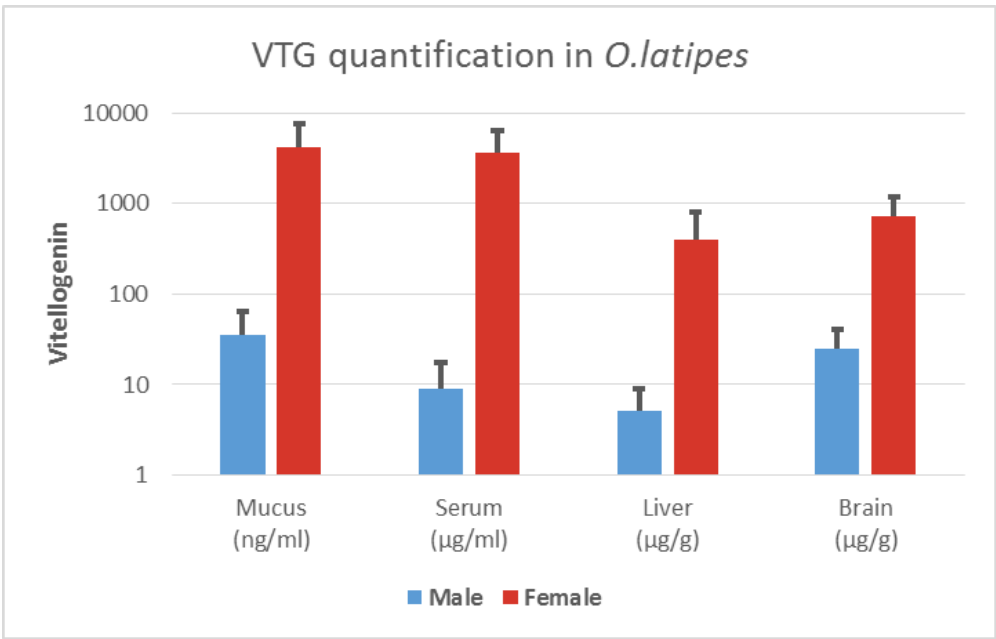
The TECO® Vitellogenin assays have been validated using different sample types. Results from different laboratory exposure experiments and experiments in the field are presented below.

Vitellogenin levels in different sample types from male and female fish:

TECO® REACH Medaka Vitellogenin ELISA

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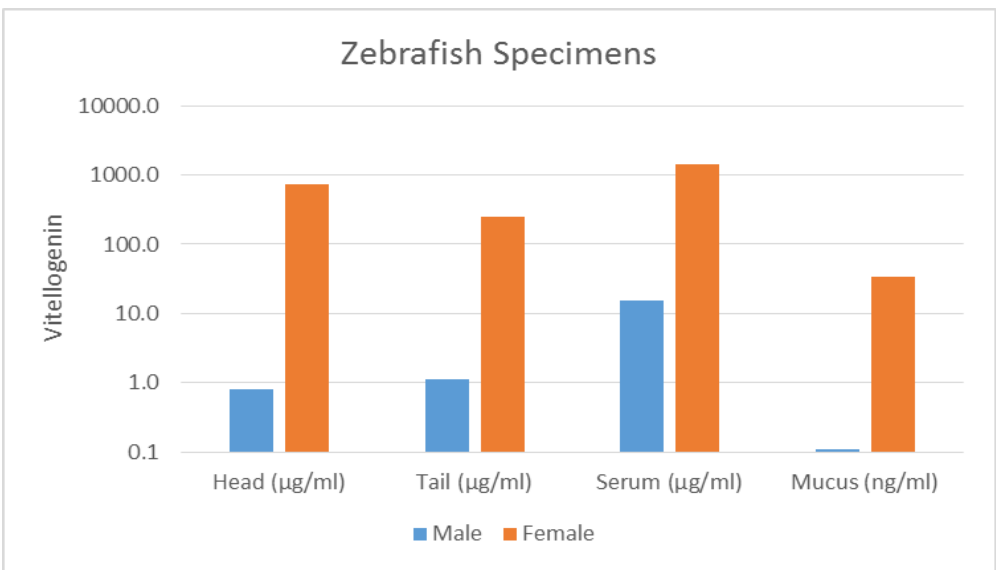
Japanese rice fish: VTG level in different sample types (mucus vs. serum vs. homogenate)



TECO® Cyprinid Vitellogenin ELISA

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Male and female zebrafish: VTG level in different sample types (mucus vs. serum vs. homogenate)

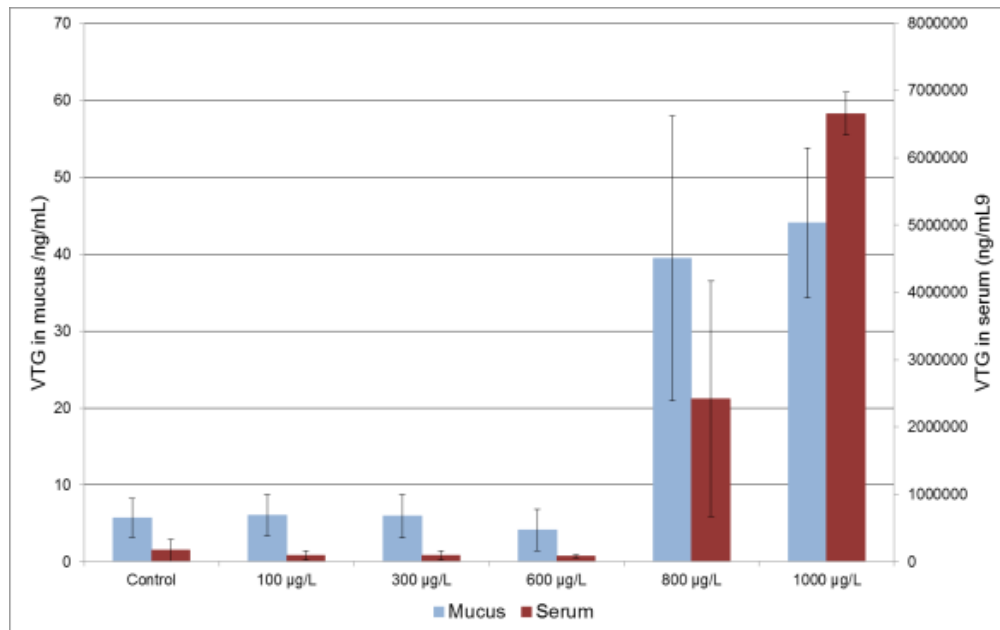


Development of vitellogenin levels in different exposure experiments:

TECO® Cyprinid Vitellogenin ELISA

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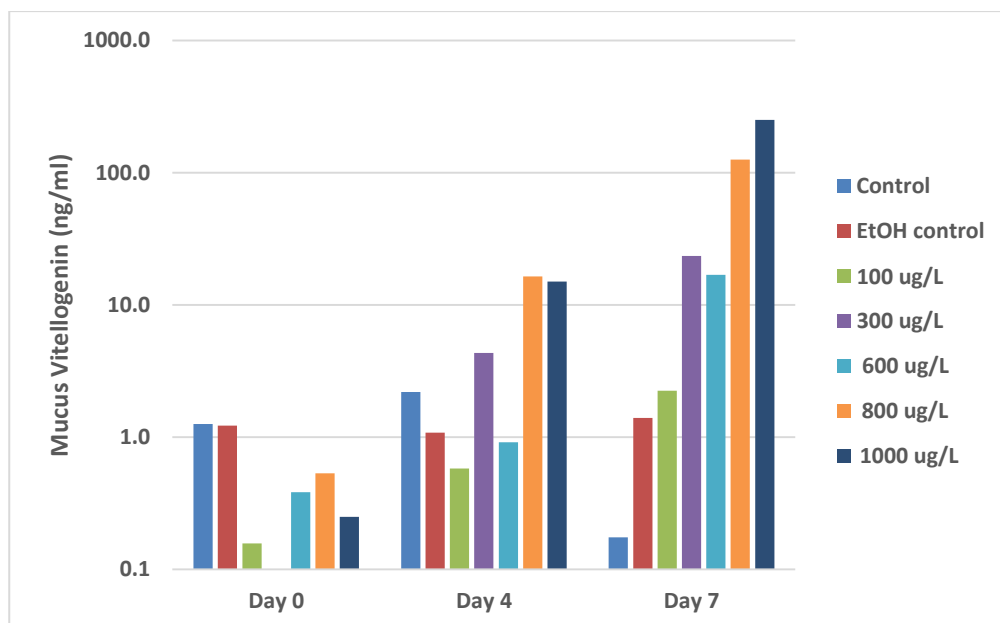
Zebrafish (n=7) exposure experiment using BPA



TECO® Cyprinid Vitellogenin ELISA

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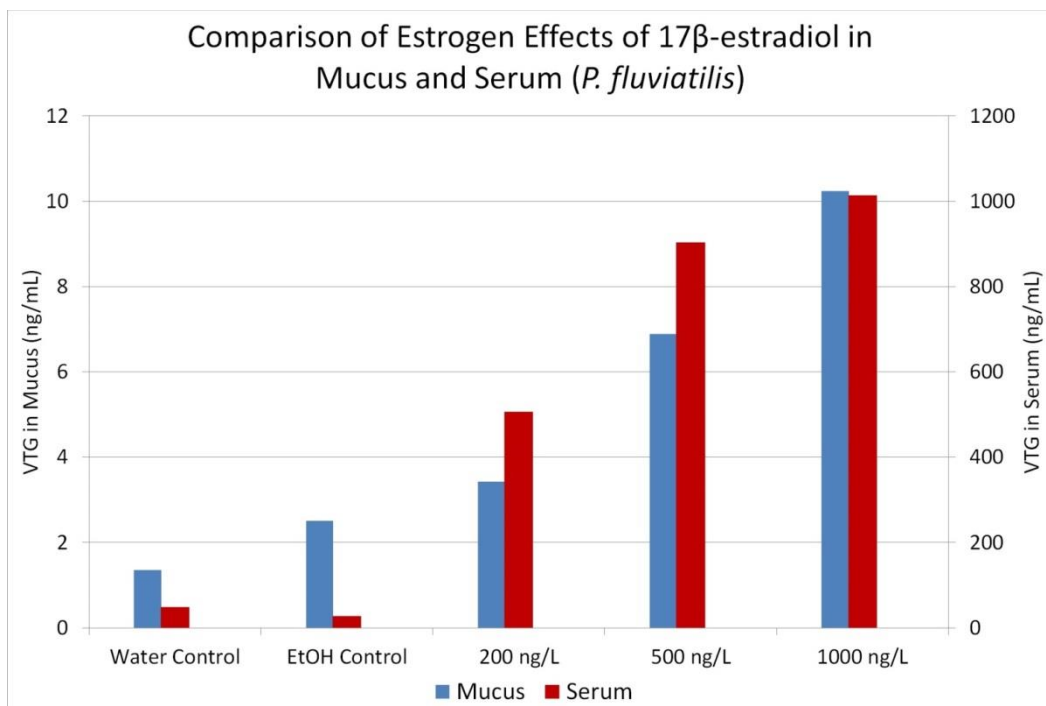
Fathead Minnow (n=5-7/group) 7-day exposure experiment using BPA



TECO® Perch (Perciformes) Vitellogenin ELISA

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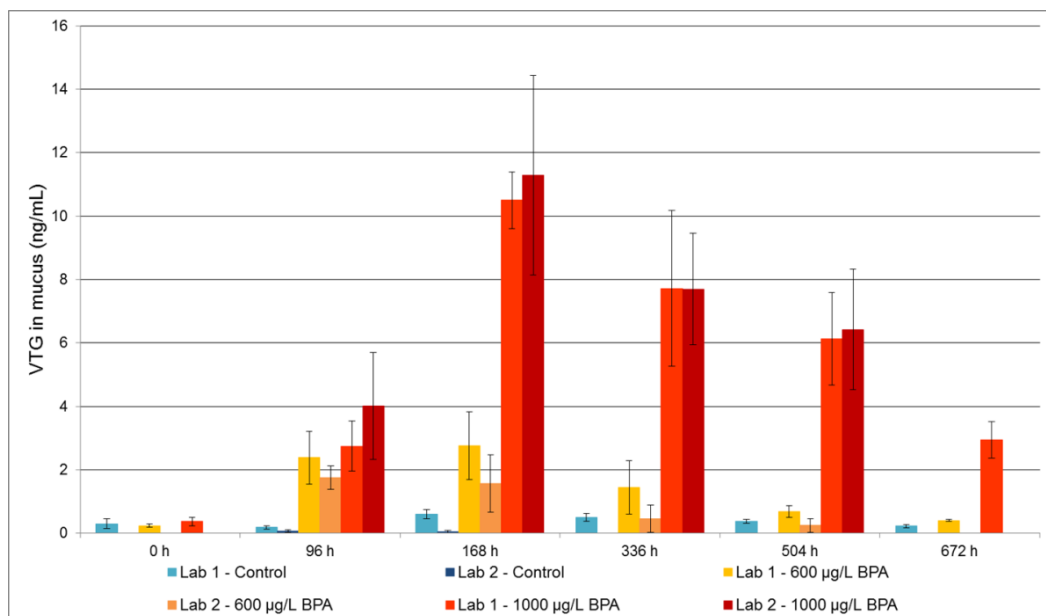
European Perch exposure experiment using E2: VTG concentrations in serum & mucus at day 6



TECO® Perch (Perciformes) Vitellogenin ELISA

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Bluegill 28-day exposure experiment using BPA: 2 swabs/fish & samples analyzed in two independent labs

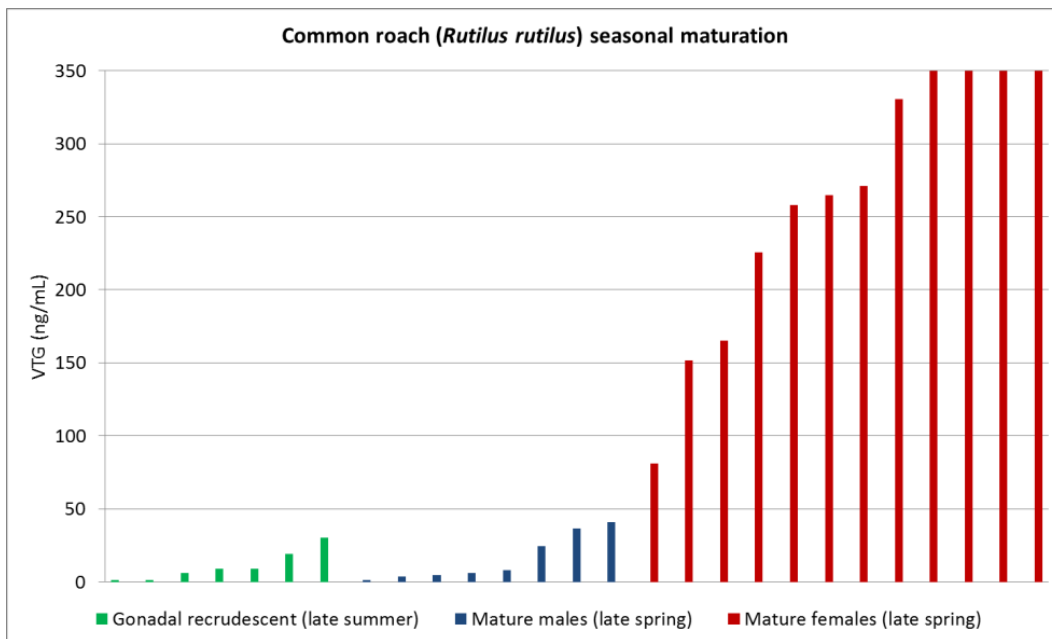


Vitellogenin measurements in habitat:

TECO® Cyprinid Vitellogenin ELISA

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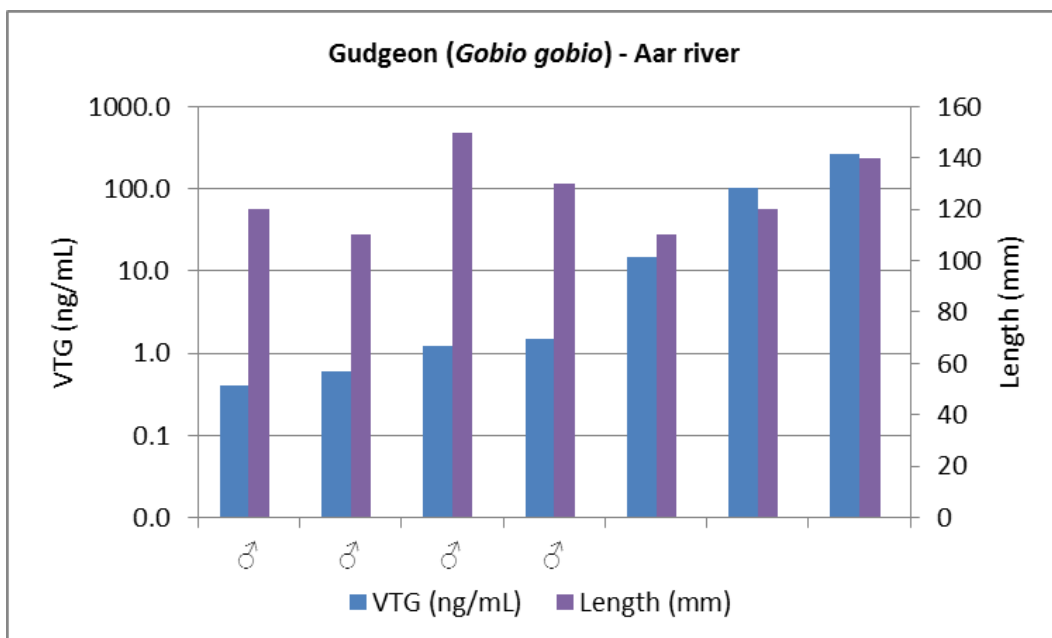
Common Roach: vitellogenin determination in mucus in different seasons - sex differentiation possible in late spring.



TECO® Cyprinid Vitellogenin ELISA

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Gudgeon: sex differentiation using length & mucus vitellogenin concentration



Crossreactivities in TECO® Vitellogenin-ELISAs

Name	Species	Family	Crossreaction
Vitellogenin Cyprinid TECO - Cat. No.: TE1037			
Carp	Cyprinus carpio	Cyprinidae	+++
Goldfish	Carassius gibelio auratus	Cyprinidae	+++
Zebrafish	Danio rerio	Cyprinidae	++
Fathead minnow	Pimephales promelas	Cyprinidae	++
"Common bream/freshwater bream/bronze bream/carp bream"	Abramis brama	Cyprinidae	+++
Roach	Rutilus rutilus	Cyprinidae	+++
Common rudd	Scardinius erythrophthalmus	Cyprinidae	+++
Chub	Squalius cephalus	Cyprinidae	+++
Common nase	Chondrostoma nasus	Cyprinidae	+++
Bleak	Alburnus alburnus	Cyprinidae	++
Neon Tetra	Paracheirodon innesi	Characidae	+
Gudgeon	Gobio gobio	Cyprinidae	+++
Common dace	Leuciscus leuciscus	Cyprinidae	+++
Stone loach	Barbatula barbatula	Nemacheilidae	++
Common minnow	Phoxinus phoxinus	Cyprinidae	+++
Vitellogenin Cyprinid TECO REACH - Cat. No.: TE1040			
Carp	Cyprinus carpio	Cyprinidae	+++
Goldfish	Carassius gibelio auratus	Cyprinidae	+++
Zebrafish	Danio rerio	Cyprinidae	++
Fathead minnow	Pimephales promelas	Cyprinidae	++
Vitellogenin Cyprinid TECO Ultra-Sensitive - Cat. No.: TE1046			
Carp	Cyprinus carpio	Cyprinidae	+++
Goldfish	Carassius gibelio auratus	Cyprinidae	+++
Zebrafish	Danio rerio	Cyprinidae	++
Fathead minnow	Pimephales promelas	Cyprinidae	++
"Common bream/freshwater bream/bronze bream/carp bream"	Abramis brama	Cyprinidae	+++
Roach	Rutilus rutilus	Cyprinidae	+++
Common rudd	Scardinius erythrophthalmus	Cyprinidae	+++
Chub	Squalius cephalus	Cyprinidae	+++
Common nase	Chondrostoma nasus	Cyprinidae	+++
Bleak	Alburnus alburnus	Cyprinidae	++
Neon Tetra	Paracheirodon innesi	Characidae	+
Gudgeon	Gobio gobio	Cyprinidae	+++
Common dace	Leuciscus leuciscus	Cyprinidae	+++
Stone loach	Barbatula barbatula	Nemacheilidae	++
Common minnow	Phoxinus phoxinus	Cyprinidae	+++

+++ high (close to 100%)
 ++ medium
 + useful in exposition experiments
 0 weak

Crossreactivities in TECO® Vitellogenin-ELISAs

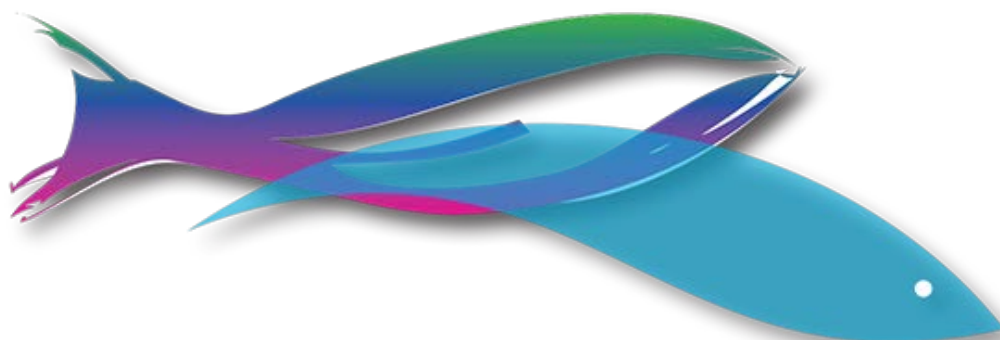
Vitellogenin Perch (Perciformes) TECO - Cat. No.: TE1035			
Tilapia	Oreochromis niloticus	Cichlidae	+++
Bluegill	Lepomis macrochirus	Centrarchidae	+
"European perch/perch/redfin perch/English perch"	Perca fluviatilis	Percidae	+
Ruffe	Gymnocephalus cernua	Percidae	+
Goby	Neogobius sp.	Gobiidae	+
Three-spined stickleback	Gasterosteus aculeatus	Gasterosteidae	+
Vitellogenin Perch (Perciformes) TECO REACH - Cat. No.: TE1039			
Tilapia	Oreochromis niloticus	Cichlidae	+++
Bluegill	Lepomis macrochirus	Centrarchidae	+
Vitellogenin Multi Species TECO - Cat. No.: TE1042			
Japanese rice fish	Oryzias latipes	Adrianichthyidae	+++
Rainbow fish	Melanotaenia praecox	Melanotaeniidae	+++
Atlantic cod	Gadus morhua	Gadidae	++
Common dab	Limanda limanda	Pleuronectidae	++
European plaice	Pleuronectes platessa	Pleuronectidae	++
European flounder	Platichthys flesus	Pleuronectidae	++
Atlantic herring	Clupea harengus	Clupeidae	+
Tuna	Thunnus spec.	Scombridae	++
Vitellogenin Medaka TECO REACH - Cat. No.: TE1043			
Japanese rice fish	Oryzias latipes	Adrianichthyidae	+++
Vitellogenin Salmonid TECO - Cat. No.: TE1047			
Atlantic salmon	Salmo salar	Salmonidea	+++
Brown trout	Salmo trutta	Salmonidea	+++
Chum salmon	Oncorhynchus keta	Salmonidea	+++
Pink salmon/humpback salmon	Oncorhynchus gorbuscha	Salmonidea	+++
Rainbow trout	Oncorhynchus mykiss	Salmonidea	+++
Brook trout	Salvelinus fontinalis	Salmonidea	+++
"Common whitefish, European whitefish"	Coregonus lavaretus	Salmonidea	+++*
Maraena whitefish	Coregonus maraena	Salmonidea	+++*
Vitellogenin Salmonid TECO Ultra Sensitive - Cat. No.: TE1049			
Atlantic salmon	Salmo salar	Salmonidea	+++
Brown trout	Salmo trutta	Salmonidea	+++
Chum salmon	Oncorhynchus keta	Salmonidea	+++
Pink salmon/humpback salmon	Oncorhynchus gorbuscha	Salmonidea	+++
Rainbow trout	Oncorhynchus mykiss	Salmonidea	+++
Brook trout	Salvelinus fontinalis	Salmonidea	+++
"Common whitefish, European whitefish"	Coregonus lavaretus	Salmonidea	+++*
Maraena whitefish	Coregonus maraena	Salmonidea	+++*

+++ high (close to 100%)
 ++ medium
 + useful in exposition experiments
 0 weak

*VTG instable

3 Types of Vitellogenin ELISA protocols:

- **Standard** Vitellogenin ELISAs for different species,
- **REACH** test kit ELISAs to meet regulatory requirements, or
- **Ultra-Sensitive** Vitellogenin ELISA kits to measure very low protein concentrations



TECO[®] Cyprinid Vitellogenin ELISA (TE1037)

For **Research Use Only**

The Cyprinid Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of cyprinids.

Standards	Standard Stock, lyophilized, 2 vials
Controls	3 Control levels, lyophilized, 2 vials each
Standard Range	0.4-35 ng/ml
Extended Range	0.4-70 ng/ml
Sensitivity:	
LLOQ	0.4 ng/ml
LLD	0.036 ng/ml
Incubation time	4.0 hours
Sample volume	50 µL
Species	Carp (<i>Cyprinus carpio</i>); Goldfish (<i>Carassius gibelio auratus</i>); Zebrafish (<i>Danio rerio</i>); Fathead minnow (<i>Pimephales promelas</i>); "Common bream/freshwater bream/bronze bream/carp bream" (<i>Abramis brama</i>); Roach (<i>Rutilus rutilus</i>); Common rudd (<i>Scardinius erythrophthalmus</i>); Chub (<i>Squalius cephalus</i>); Common nase (<i>Chondrostoma nasus</i>); Bleak (<i>Alburnus alburnus</i>); Neon Tetra (<i>Paracheirodon innesi</i>); Gudgeon (<i>Gobio gobio</i>); Common dace (<i>Leuciscus leuciscus</i>); Stone loach (<i>Barbatula barbatula</i>); Common minnow (<i>Phoxinus phoxinus</i>).

TECO[®] REACH Cyprinid Vitellogenin ELISA (TE1040)

For Research Use Only

The REACH Cyprinid Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of cyprinids according to EC regulation No. 440/2008 (REACH) from July 10th 2015/ Document D039048/03.

Standards	Standard Stock, lyophilized, 2 vials
Controls	3 Control levels, lyophilized, 2 vials each
NSB-Strip	Inter-Assay Reference Standard Stock, lyophilized, 1 vial 1 breakable strip of 8 wells
Standard Range	0.4-35 ng/ml
Extended Range	0.4-70 ng/ml
Sensitivity:	
LLOQ	0.4 ng/ml
LLD	0.036 ng/ml
Incubation time	4.0 hours
Sample volume	50 µL
Species	Carp (<i>Caprinus carpio</i>); Goldfish (<i>Carassius auratus</i>); Zebrafish (<i>Danio rerio</i>); Fathead Minnow (<i>Pimephales promelas</i>).

TECO[®] Ultra Sensitive Cyprinid Vitellogenin ELISA (TE1046)

For Research Use Only

The Ultra Sensitive Cyprinid Vitellogenin ELISA Kit is a very sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in samples obtained from cell culture and mucus of cyprinids.

Standards	Standard Stock, lyophilized, 2 vials
Controls	2 Control levels, lyophilized, 2 vials each
Range	0.025 -2.0 ng/mL
Sensitivity:	
LLOQ	< 0.025 ng/ml
LLD	0.002 ng/ml
Incubation time	Overnight 16-24 hours plus 4h±10 min
Sample volume	50 µL
Species	Carp (<i>Cyprinus carpio</i>); Goldfish (<i>Carassius gibelio auratus</i>); Zebrafish (<i>Danio rerio</i>); Fathead minnow (<i>Pimephales promelas</i>); "Common bream/freshwater bream/bronze bream/carp bream" (<i>Abramis brama</i>); Roach (<i>Rutilus rutilus</i>); Common rudd (<i>Scardinius erythrophthalmus</i>); Chub (<i>Squalius cephalus</i>); Common nase (<i>Chondrostoma nasus</i>); Bleak (<i>Alburnus alburnus</i>); Neon Tetra (<i>Paracheirodon innesi</i>); Gudgeon (<i>Gobio gobio</i>); Common dace (<i>Leuciscus leuciscus</i>); Stone loach (<i>Barbatula barbatula</i>); Common minnow (<i>Phoxinus phoxinus</i>).

TECO[®] Perch (Perciformes) Vitellogenin ELISA (TE1035)

For Research Use Only

The Perch (Perciformes) Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of perciformes.

Standards	Standard Stock, 2 vials
Controls	2 Control levels, 2 vials each
Range	1-80 ng/mL
Sensitivity:	
LLOQ	< 1.0 ng/ml
LLD	< 0.22 ng/ml
Incubation time	4.0 hours
Sample volume	50 µL
Species	Tilapia (<i>Oreochromis niloticus</i>); Bluegill (<i>Lepomis macrochirus</i>); "European perch/perch/redfin perch/English perch" (<i>Perca fluviatilis</i>); Ruffe (<i>Gymnocephalus cernua</i>); Three-spined stickleback (<i>Gasterosteus aculeatus</i>)

TECO[®] REACH Perch (Perciformes) Vitellogenin ELISA (TE1035)

For Research Use Only

The Perch (Perciformes) Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of perciformes according to EC regulation No. 440/2008 (REACH) from July 10th 2015/ Document D039048/03.

Standards	Standard Stock, 2 vials
Controls	2 Control levels, 2 vials each
	Inter-Assay Reference Standard Stock, lyophilized, 1 vial
NSB-Strip	1 breakable strip of 8 wells
Range	1-80 ng/mL
Sensitivity:	
LLOQ	< 1.0 ng/ml
LLD	< 0.22 ng/ml
Incubation time	4.0 hours
Sample volume	50 µL
Species	Tilapia (<i>Oreochromis niloticus</i>); Bluegill (<i>Lepomis macrochirus</i>); "European perch/perch/redfin perch/English perch" (<i>Perca fluviatilis</i>); Ruffe (<i>Gymnocephalus cernua</i>); Three-spined stickleback (<i>Gasterosteus aculeatus</i>)

TECO[®] REACH Medaka Vitellogenin ELISA (TE1043)

For Research Use Only

The REACH Medaka Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of medaka according to EC regulation No. 440/2008 (REACH) from July 10th 2015/ Document D039048/03.

Standards	Standard Stock, lyophilized, 2 vials
Controls	2 Control levels, lyophilized, 2 vials each
NSB-Strip	Inter-Assay Reference Standard Stock, lyophilized, 1 vial
Range	1 breakable strip of 8 wells
Sensitivity:	2.6 -210 ng/mL
LLOQ	< 2.6 ng/ml
LLD	0.7 ng/ml
Incubation time	4.0 hours
Sample volume	50 µL
Species	Medaka (<i>Oryzias latipes</i>)

TECO[®] Multi Species Vitellogenin ELISA (TE1042)

For Research Use Only

The Multi Species Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of different species.

Standards	Standard Stock, lyophilized, 2 vials
Controls	2 Control levels, lyophilized, 2 vials each
Range	2.6 -210 ng/mL
Sensitivity:	
LLOQ	< 2.6 ng/ml
LLD	0.7 ng/ml
Sample volume	50 µL
Incubation time	4.0 hours
Species	Japanese rice fish (<i>Oryzias latipes</i>); Australian rainbowfish (<i>Melanotaenia praecox</i>); Atlantic cod (<i>Gadus morhua</i>); Common dab (<i>Limanda limanda</i>); European plaice (<i>Pleuronectes platessa</i>); European flounder (<i>Platichthys flesus</i>); Atlantic herring (<i>Clupea harengus</i>); Tuna (<i>Thunnus spec.</i>)

TECO[®] Salmonid Vitellogenin ELISA (TE1047)

For Research Use Only

The Salmonid Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH and mucus of salmonids.

Standards	Standard Stock, lyophilized, 2 vials
Controls	2 Control levels, lyophilized, 2 vials each
Range	0.4-35 ng/mL
Sensitivity:	
LLOQ	< 0.4 ng/ml
LLD	< 0.1ng/ml
Incubation time	3.0 hours
Sample volume	50 µL
Species	Atlantic salmon (<i>Salmo salar</i>); Brown trout (<i>Salmo trutta</i>); Chum salmon (<i>Oncorhynchus keta</i>); Pink salmon/humpback salmon (<i>Oncorhynchus gorbuscha</i>); Rainbow trout (<i>Oncorhynchus mykiss</i>); Brook trout (<i>Salvelinus fontinalis</i>); "Common whitefish, European whitefish" (<i>Coregonus lavaretus</i>); Maraena whitefish (<i>Coregonus maraena</i>)

TECO[®] Ultra Sensitive Salmonid Vitellogenin ELISA (TE1049)

For Research Use Only

The Ultra Sensitive Salmonid Vitellogenin ELISA Kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of vitellogenin in serum, WBH, mucus of salmonids and in samples obtained from cell culture.

Standards	Standard Stock, lyophilized, 2 vials
Controls	2 Control levels, lyophilized, 2 vials each
Range	0.012-1.0 ng/mL
Sensitivity:	
LLOQ	0.012 ng/ml
LLD	0.002 ng/ml
Sample volume	50 µL
Incubation time	4.0 hours
Species	Atlantic salmon (<i>Salmo salar</i>); Brown trout (<i>Salmo trutta</i>); Chum salmon (<i>Oncorhynchus keta</i>); Pink salmon/humpback salmon (<i>Oncorhynchus gorbuscha</i>); Rainbow trout (<i>Oncorhynchus mykiss</i>); Brook trout (<i>Salvelinus fontinalis</i>); "Common whitefish, European whitefish" (<i>Coregonus lavaretus</i>); Maraena whitefish (<i>Coregonus maraena</i>)

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[4] Moncaut, N., Lo Nostro, F., Maggese M. C. (2003)

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[5] Allner B., Gönna von der S., Griebeler E.M., Nikutowski N., Schaaf A., Stahlschmidt-Allner P. (2010)

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[6] Allner B., Hennies M., Lerche C.F., Schmidt T., Schneider K., Willner M., Stahlschmidt-Allner P. (2016)

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