TECOmedical Group

TECO® Vitellogenin ELISA System in Fish

The world first test application in epidermal mucus

Repeated, non-invasive, non-destructive sampling

Following the “Three R’s principle” (Replacement, Reduction, Refinement)

Sampling method of choice for outdoors projects

Broad range of Vitellogenin ELISAs

Vitellogenin ELISAs for different species

REACH test kit configurations to cover regulatory requirements

Ultra Sensitive Vitellogenin ELISA kits

Multiple applications for TECO® Vitellogenin ELISAs

Covering more than 37 fish species (including marine fish)

Different sample types: Mucus, gill swab, blood, different homogenates

Vitellogenin determination in juvenile, male and female fish, cell culture

Chemical testing according to OECD Guidelines

Environmental and ecotoxicological testing

always your partner

Introduction

Vitellogenin in fish is an estrogen induced yolk precursor protein mainly synthesized in the liver to be deposited in the maturing oocytes, where it is split in the yolk proteins lipovitellin1, lipovitellin 2 and phosvitin. These yolk proteins serve as nourishment storage for the developing embryos. Due to the estrogen-depending synthesis and the significance in the nutrition of the offspring, vitellogenin is considered as a typical “female protein”.

As male and juvenile fish do hardly produce any estrogens, the vitellogenin levels are quiet low. Non-physiological induction of vitellogenin in these 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 cleavable precursor of the yolk proteins (see above) and therefore a very instable protein. For this reason samples have to be frozen immediately after sampling.

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, in particular where very small fish are concerned, or in approaches 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 organize in ecotoxicological studies outdoors.

Recently different studies have shown, that vitellogenin can also be determined 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 as it includes a validated mucus collection set for gentle and effective mucus sampling and also adapted high sensitive 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)

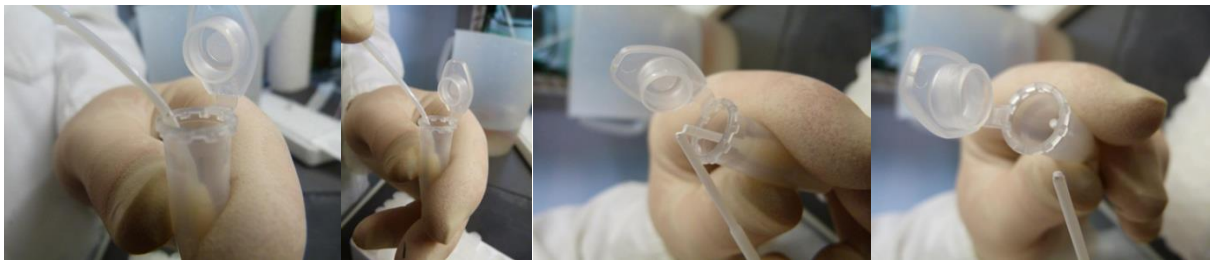
The Mucus Collection Set includes validated swabs, sample tubes, a sample tube rack, a kit instruction and a specifically designed extraction buffer for usage in TECO® Vitellogenin ELISAs. The Mucus Collection Set is a validated tool for repeatable non-invasive/non-destructive sampling of epidermal mucosa 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 in epidermal mucus using the TECO® Mucus Collection Set

- ✓ **Cortisol:** Stress marker (TECO®Mucus-Cortisol EIA, TE 1052)
- ✓ **Hyaluronic Acid:** Possible marker for liver toxicity (specific protocol for mucus application in TECO®Hyaluronic Acid ELISA, TE 1018-2)

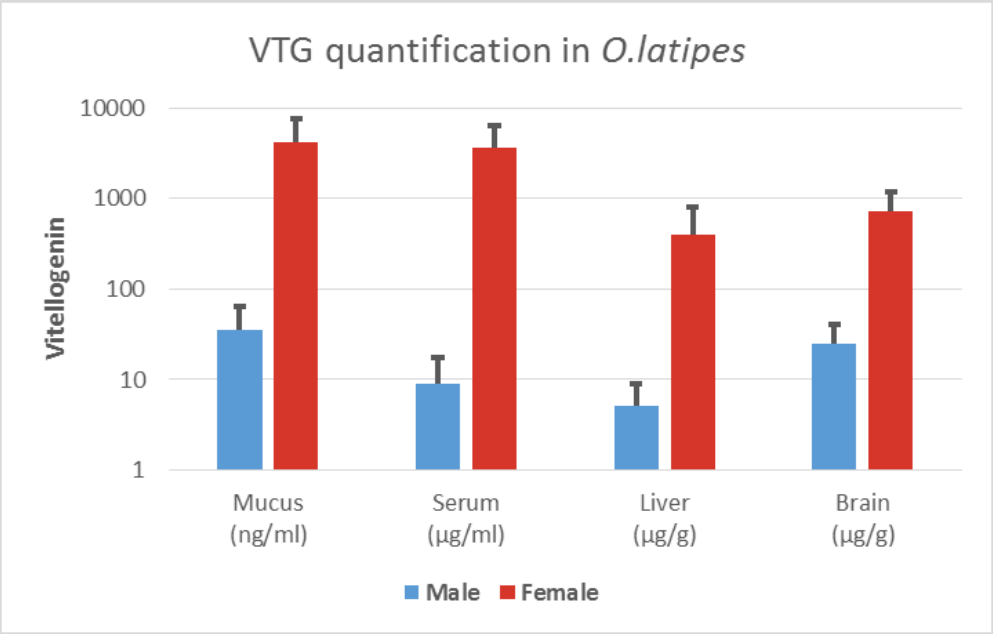
TECO® Vitellogenin ELISA

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

Vitellogenin level in different sample types of male and female fish

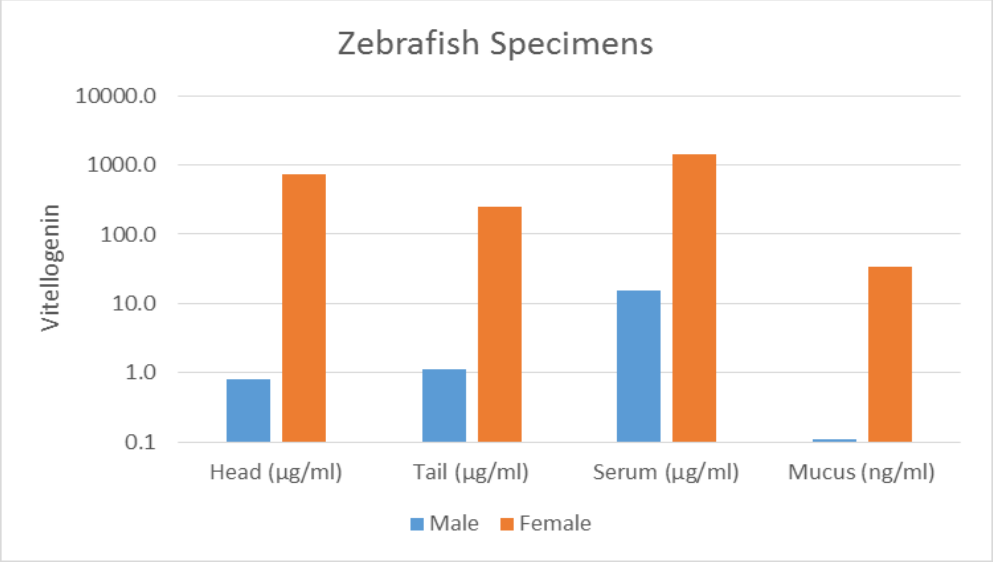
TECO® *REACH* Medaka Vitellogenin ELISA

VTG level in different sample types in the Japanese rice fish



TECO® Cyprinid Vitellogenin ELISA

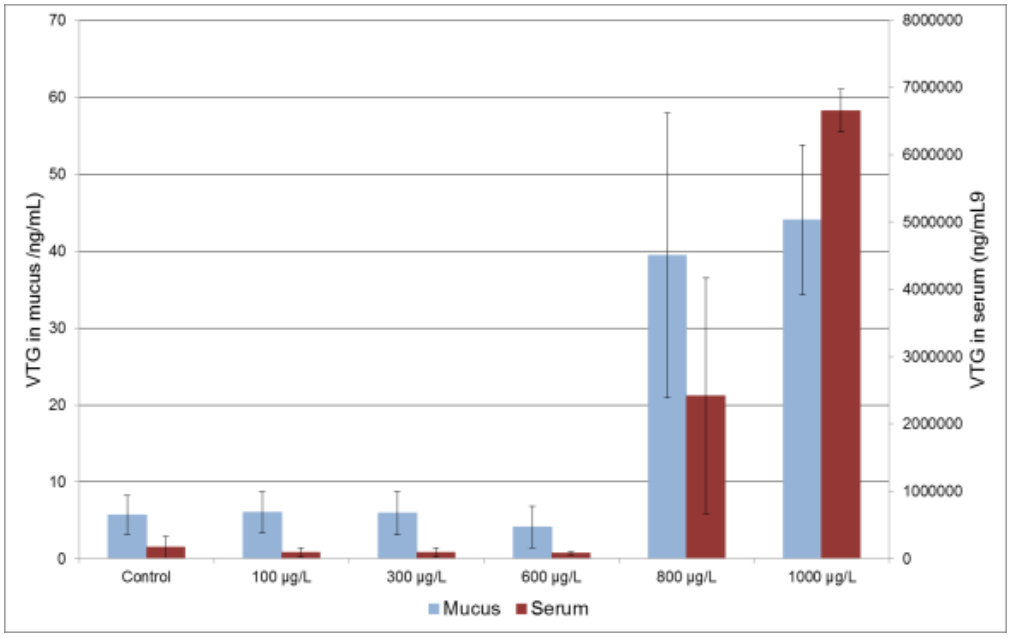
Male and female zebrafish: different sample types



Development of vitellogenin levels in different exposition experiments

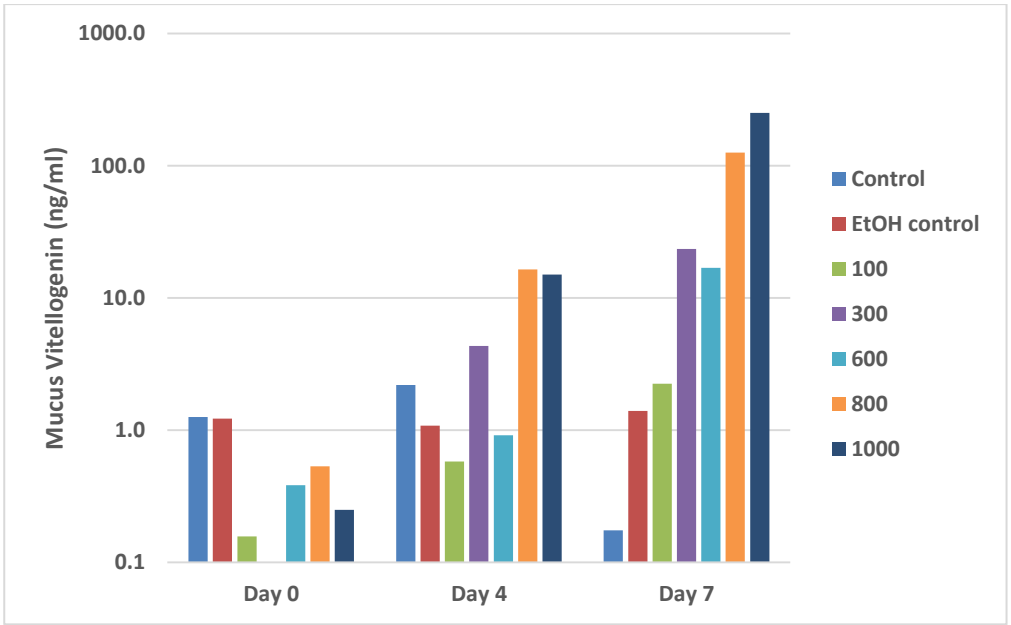
TECO® Cyprinid Vitellogenin ELISA

Exposition experiment with BPA using zebrafish (n=7)



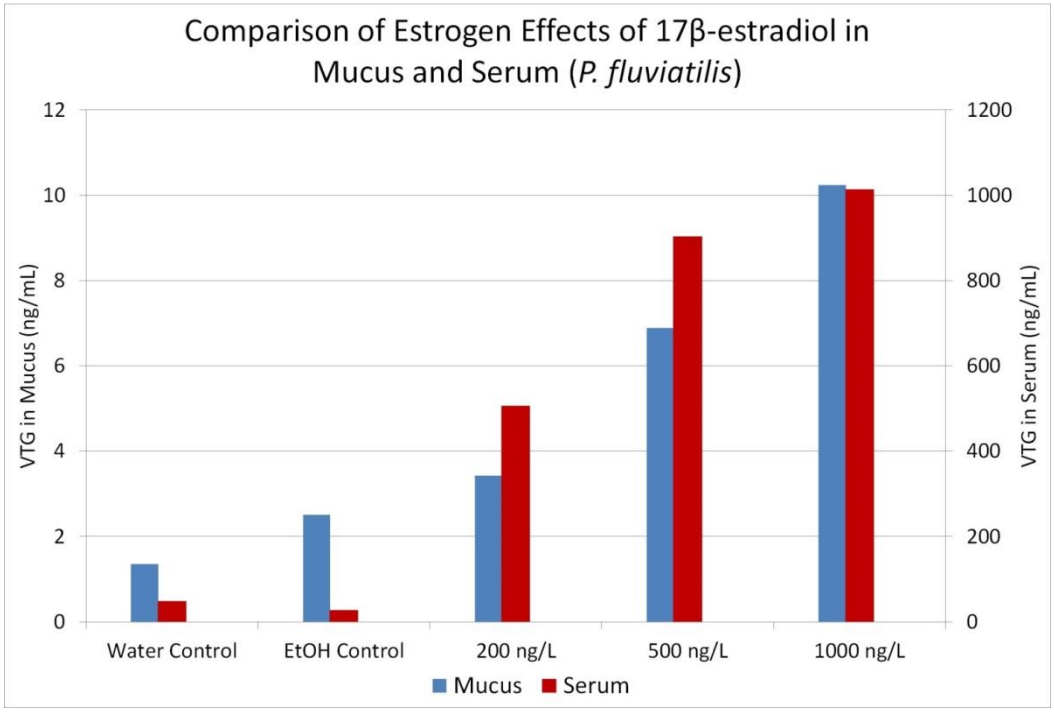
TECO® Cyprinid Vitellogenin ELISA

7 day exposition experiment with BPA using fathead minnow (n=5-7/group)



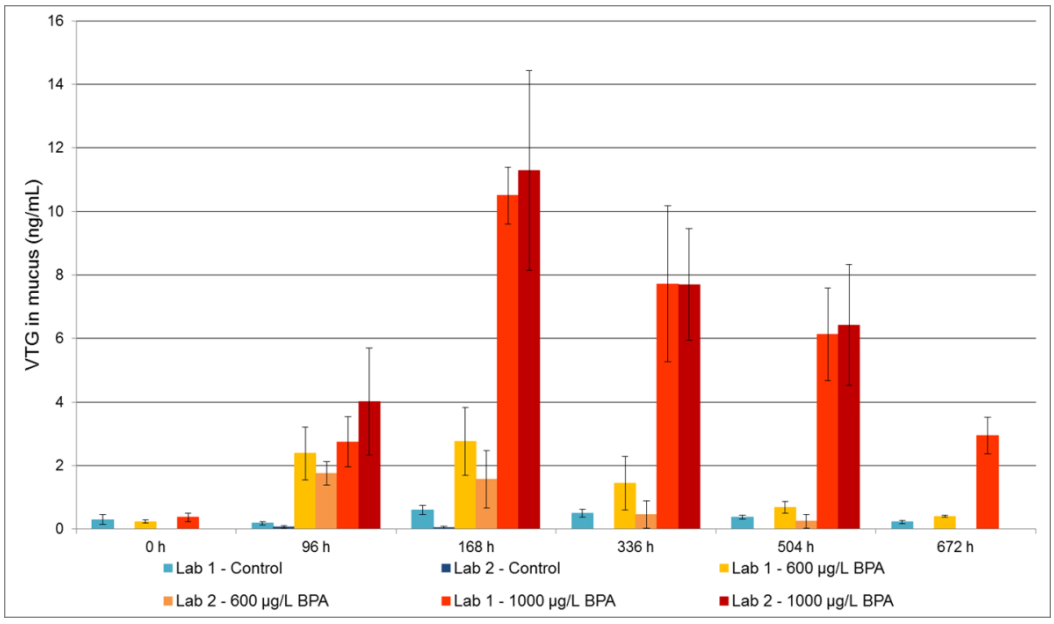
TECO® Perch (Perciformes) Vitellogenin ELISA

Exposition experiment with E2 in serum and mucus in European Perch at day 6



TECO® Perch (Perciformes) Vitellogenin ELISA

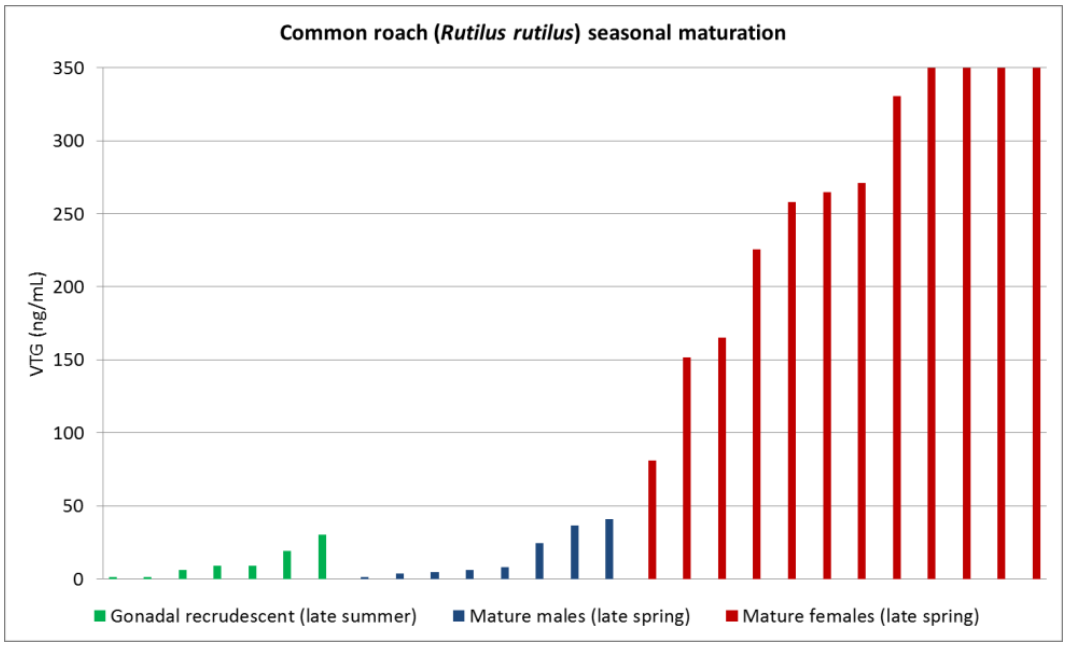
Long-term BPA Exposition using bluegill (2 swabs/fish; samples were analyzed in two independent laboratories)



Vitellogenin measurements in habitat

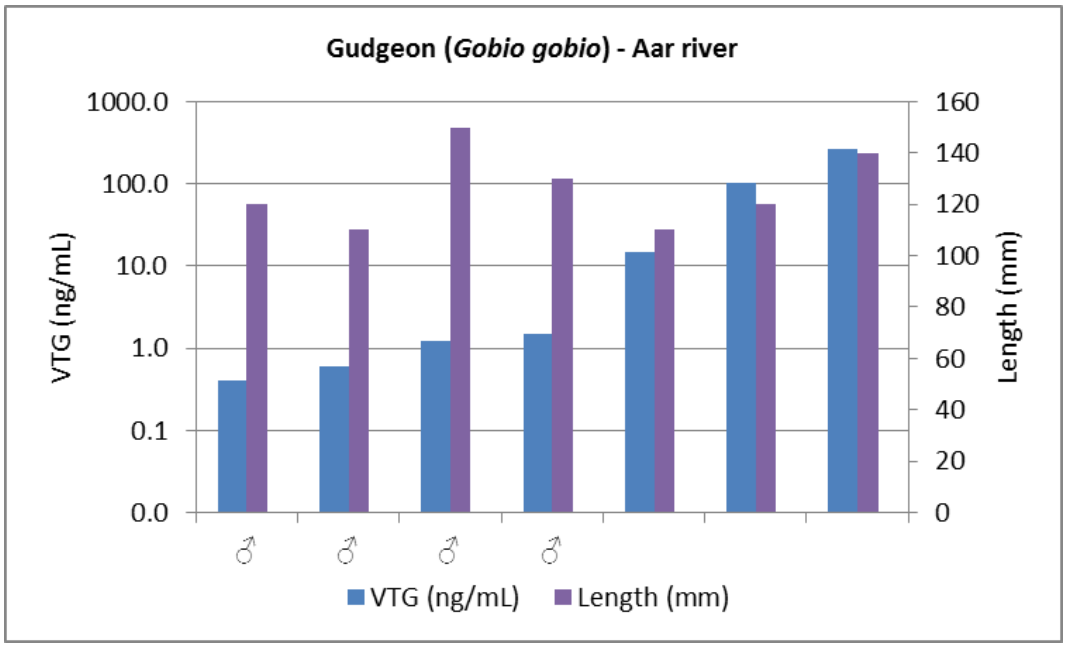
TECO® Cyprinid Vitellogenin ELISA

Common roach: Vitellogenin determination in mucus in different seasons -sex differentiation possible in late spring.



TECO® Cyprinid Vitellogenin ELISA

Gudgeon: Sex differentiation using length and vitellogenin concentration in mucus



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%)
++	mediuml
+	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	+++*

*VTG instable

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

TECO® Vitellogenin ELISAs

- ✓ Developed and produced under ISO 13485 und ISO 9001 standards;
- ✓ Minimum shelf life from production of 12 months;
- ✓ Test kits are designed for two assay runs per test kits;
- ✓ Test kits contain minimum of two controls;
- ✓ Sample preparation and assay run at room temperature;
- ✓ Buffer system allows parallel measurement of protein concentration in samples;
- ✓ Multiple sample types: blood, homogenates (head, tail, liver, brain), epidermal mucus and gill swab samples.

TECO® Cyprinid Vitellogenin ELISA (TE1037)

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 (*Cyprinus carpio*); Goldfish (*Carassius gibelio auratus*); Zebrafish (*Danio rerio*); Fathead minnow (*Pimephales promelas*); "Common bream/freshwater bream/bronze bream/carp bream" (*Abramis brama*); Roach (*Rutilus rutilus*); Common rudd (*Scardinius erythrophthalmus*); Chub (*Squalius cephalus*); Common nase (*Chondrostoma nasus*); Bleak (*Alburnus alburnus*); Neon Tetra (*Paracheirodon innesi*); Gudgeon (*Gobio gobio*); Common dace (*Leuciscus leuciscus*); Stone loach (*Barbatula barbatula*); Common minnow (*Phoxinus phoxinus*).

TECO® *REACH* Cyprinid Vitellogenin ELISA (TE1040)

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
	Inter-Assay Reference Standard Stock, lyophilized, 1 vial
NSB-Strip	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 (*Caprinus carpio*); Goldfish (*Carassius auratus*); Zebrafish (*Danio rerio*); Fathead Minnow (*Pimephales promelas*).

TECO® Ultra Sensitive Cyprinid Vitellogenin ELISA (TE1046)

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 (*Cyprinus carpio*); Goldfish (*Carassius gibelio auratus*); Zebrafish (*Danio rerio*); Fathead minnow (*Pimephales promelas*); "Common bream/freshwater bream/bronze bream/carp bream" (*Abramis brama*); Roach (*Rutilus rutilus*); Common rudd (*Scardinius erythrophthalmus*); Chub (*Squalius cephalus*); Common nase (*Chondrostoma nasus*);Bleak (*Alburnus alburnus*); Neon Tetra (*Paracheirodon innesi*); Gudgeon (*Gobio gobio*); Common dace (*Leuciscus leuciscus*); Stone loach (*Barbatula barbatula*); Common minnow (*Phoxinus phoxinus*).

TECO® Perch (Perciformes) Vitellogenin ELISA (TE1035)

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 (*Oreochromis niloticus*); Bluegill (*Lepomis macrochirus*); "European perch/perch/redfin perch/English perch" (*Perca fluviatilis*); Ruffe (*Gymnocephalus cernua*); Three-spined stickleback (*Gasterosteus aculeatus*)

TECO® *REACH* Perch (Perciformes) Vitellogenin ELISA (TE1039)

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 (*Oreochromis niloticus*); Bluegill (*Lepomis macrochirus*); "European perch/perch/redfin perch/English perch" (*Perca fluviatilis*); Ruffe (*Gymnocephalus cernua*); Three-spined stickleback (*Gasterosteus aculeatus*)

TECO®*REACH* Medaka Vitellogenin ELISA (TE1043)

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

TECO® Multi Species Vitellogenin ELISA (TE1042)

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 (*Oryzias latipes*); Australian rainbowfish (*Melanotaenia praecox*); Atlantic cod (*Gadus morhua*); Common dab (*Limanda limanda*); European plaice (*Pleuronectes platessa*); European flounder (*Platichthys flesus*); Atlantic herring (*Clupea harengus*); Tuna (*Thunnus spec*.)

TECO® Salmonid Vitellogenin ELISA (TE1047)

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 (*Salmo salar*); Brown trout (*Salmo trutta*); Chum salmon (*Oncorhynchus keta*); Pink salmon/humpback salmon (*Oncorhynchus gorbuscha*); Rainbow trout (*Oncorhynchus mykiss*); Brook trout (*Salvelinus fontinalis*); "Common whitefish, European whitefish" (*Coregonus lavaretus*); Maraena whitefish (*Coregonus maraena*)

TECO® Ultra Sensitive Salmonid Vitellogenin ELISA (TE1049)

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 (*Salmo salar*); Brown trout (*Salmo trutta*); Chum salmon (*Oncorhynchus keta*); Pink salmon/humpback salmon (*Oncorhynchus gorbuscha*); Rainbow trout (*Oncorhynchus mykiss*); Brook trout (*Salvelinus fontinalis*); "Common whitefish, European whitefish" (*Coregonus lavaretus*); Maraena whitefish (*Coregonus maraena*)

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