TECOmedical Group

TECO®
Mucus Collection Set

Mucus Collection Set

Patent submitted (PCT/DE2014/100161)

Instructions for use English

Catalogue No. TE 1034
For Research Use Only

Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

Intended use

TE 1034

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TECO® Mucus Collection Set:

CONT Reagents and Materials Supplied:

SYMBOL	DESCRIPTION	FORMAT
1	Breakable Swabs Ready to use.	42
A	Reaction Vials Ready to use.	42
B	Extraction Buffer Ready to use.	1 x 25 mL
C	Rack for Reaction Vials	1
	Kit instructions	1 x

Storage

The kit has to be stored at 2-8 °C until expiry date. Do not freeze. Store unused reagents at 2-8 °C.

Intended Use

The TECO® Mucus Collection Set for fish mucus is a validated tool for repeatable non-destructive sampling of epidermal mucosa for vitellogenin determination and designed as an accessory kit to be used exclusively with the different TECO® ELISA kits:

- TECO® Perch (Perciformes) Vitellogenin ELISA Kit TE1035
- TECO® REACH Perch (Perciformes) Vitellogenin ELISA Kit TE1039
- TECO® Cyprinid Vitellogenin ELISA Kit TE1037
- TECO® REACH Cyprinid Vitellogenin ELISA Kit TE1040
- TECO® Ultra Sensitive Cyprinid Vitellogenin ELISA Kit TE1046
- TECO® REACH Medaka Vitellogenin ELISA Kit TE1043
- TECO® Multi Species Vitellogenin ELISA Kit TE1042
- TECO® Salmonid Vitellogenin ELISA Kit TE1047
- TECO® Ultra Sensitive Salmonid Vitellogenin ELISA Kit TE1049

- TECO® Fish Hyaluronic Acid ELISA Kit TE1060

(Please order these kits separately)

Background

Vitellogenin (VTG) 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). Originally believed to be produced only in the liver, several cell types have recently been shown to produce VTG after estrogen stimulation, including those of the epidermal mucosa of fish (4). Given the destructive nature of traditional sampling, collecting successive samples from fish has been a widespread problem, as for instance in the analysis of vitellogenin induction while screening and testing chemical substances for endocrine disruption potential. Blood is difficult to collect, in particular where very small fish are concerned, or in approaches where the animals must survive sampling. This is particularly important in field monitoring in order to avoid impact on the population under investigation (5). Even though the VTG concentration in the skin mucus is an order of magnitude lower than in blood serum or in body homogenates (containing liver tissue), the skin mucosa is very well suited as a matrix to determine exogenous VTG induction caused by environmental chemicals with affinity to the estrogen receptors (6).

Hyaluronic acid (HA), also known as hyaluronan or hyaluronate is a large linear non-sulfated glycosaminoglycan with a molecular weight between 106 and 107 Da. It is a major component of connective tissues and thus distributed ubiquitously in the organism. About one-half of the body's entire hyaluronan is found in the skin and about one fourth in the skeleton and it's supporting structures like ligaments and joints. Hyaluronan is synthesized by fibroblasts and other specialized connective tissue cells. Hyaluronan is especially important for the structure and organization of extracellular matrices. The hyaluronan network acts as an osmotic buffer and is responsible for water homeostasis as well as it regulates protein distribution via the formation of flow and diffusion barriers.
A certain amount of hyaluronan is degraded locally, but the much larger part is removed and degraded by the lymphatic system. The remainder enters the blood circulation where it is removed primarily by liver endothelial cells. A minor portion is metabolized by the kidneys and the spleen. Increased serum levels were found either due to an excessive synthesis of hyaluronan (e.g. joint or skin disease, cancer) or due to a decreased hepatic clearance (liver fibrosis/cirrhosis).
Therefore, HA measurement is a new approach to determine changes in HA turnover in the liver (e.g. by liver toxic substances) or in the fish skin by using mucus samples.

Mucus sampling with the aid of swabs constitutes an alternative to traditional procedures, avoiding animal injury by using a highly sensitive ELISA in combination with a high quality extraction buffer and swab sampling, the determination of mucosa-derived parameters has the following advantages:

- **Non-destructive, non-invasive sampling**
- **Defined matrix without lymphatic fluid contamination**
- **Allows the use of smaller fish for testing and monitoring purposes**
- **Repeated sampling allows for individual recording of the induction kinetic**

The sampling technique leads to highly comparable results when testing samples from the left and the right side from the same individual in inter-laboratory trials.

The extraction buffer is protein free and therefore an additional protein determination in the extracted sample is always possible.

No influence on the vitellogenin values in the mucus was observed after usage of the anesthetic MS 222 (Tricaine methanesulfonate).

REFERENCES

1 **OECD (2009),Test No. 229**
Fish Short Term Reproduction Assay. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing.

2 **OECD (2009),Test No. 230**
21-day Fish Assay: A Short-Term Screening for Oestrogenic and Androgenic Activity, and Aromatase Inhibition. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing.

3 **OECD (2011),Test No. 234**
Fish Sexual Development Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing.

4 **Moncaut, N., Lo Nostro, F., Maggese M. C. (2003)**
Vitellogenin detection in surface mucus of the South American cichlid fish Cichlasoma dimerus (Heckel, 1840) induced by estradiol-17b. Effects on liver and gonads. Aquatic Toxicology 63, 127-137.

5 **Allner B., Gönna von der S., Griebeler E.M., Nikutowski N., Schaat A., Stahlschmidt-Allner P.(2010)**
Reproductive functions of wild fish as bioindicators of reproductive toxicants in the aquatic environment. ESPR Environ. Sci. Pollut. Res., 17, 505-518.

6 **Allner B., Hennies M., Lerche C.F., Schmidt T., Schneider K., Willner M., Stahlschmidt-Allner P. (2016)**
Kinetic determination of vitellogenin induction in the epidermis of cyprinid and perciform fishes: Evaluation of sensitive enzyme-linked immunosorbent assays. Environ. Toxicol. Chem., 35, 2916-2930.

Warnings and Precautions

This kit is intended for in vitro use by professional persons only.
Follow the instructions carefully.

Observe expiration dates stated on the labels. Refer to ”Materials Safety Data Sheet” for more detailed safety information.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

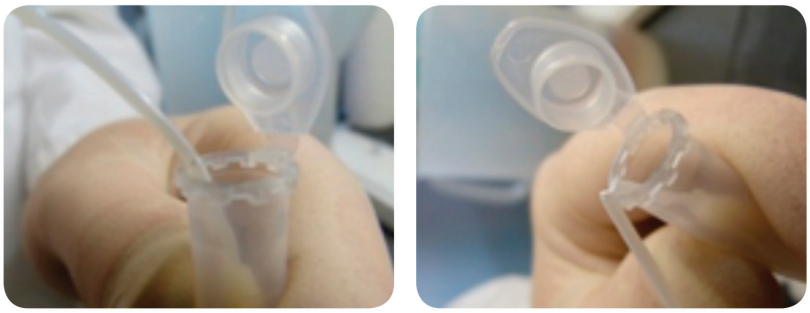
1 Treat all specimen samples as potentially biohazardous material.
Follow General Precautions when handling contents of this kit and any fish samples.
2 Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
3 Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
4 Store assay reagents as indicated.
5 A mercury-free preservative is used. Incidental contact with or ingestion of buffer solutions may cause irritation of skin, eyes or mouth. Should there be any contact, wash with water. If ingested, call a physician.

Collection of Mucus Sample

1. Prepare one collection vial per sample.
2. Place the fish carefully in position avoiding skin injuries.
3. Rub the swab gently along the body from head to tail just above the lateral line (see Figure 1a-c). Turning the swab while taking the sample.
4. Place swab into the prepared collection vial (Fig. 2a) and break the shaft at the breaking point (Figure 2b).
5. Close the collection vial and store sample at -20°C until analysis.

Figure 1a-c: Collecting fish mucus by using a swab.

Figure 2a-b: Transfer of mucus swab into collection vial.



Samples stability

Stability of mucus samples

Mucus-containing swabs from can be stored several months at < -20°C.
Avoid freeze/thaw cycles.

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