For Research Use Only

Additional information for the use of fish specimens using TECO® Hyaluronic Acid PLUS ELISA Kit

All information regarding required materials, assay procedure etc. are in the package insert. Please take the following notes into consideration, if samples from fish are used.

Preparation of standard curve and kit controls

All Hyaluronic acid (HA) Standards and Controls have to be diluted **1:50 with VTG Dilution Buffer** (instead of Sample Diluent) before pipetting into the wells (e.g. 10 µl Standard with 490 µl VTG Dilution Buffer).

Preparation of samples

Serum
Store fresh serum samples immediately after collection at -20 °C or lower until assayed. Recommended samples thawing: A simple and fast method is to place the frozen serum samples in normal tap water (15 – 20 °C). They should be thawed within 10 to 15 minutes. For assay, serum samples should be pre-diluted dependent on fish species with **VTG Dilution Buffer,** e.g. 1:20 to 1:50 (final).

Whole Body Homogenate (WBH)
Store fresh WBH samples immediately after preparation below -20 °C until assayed. For assay, WBH samples should be pre-diluted dependent on fish species with **VTG Dilution Buffer**, e.g. 1:5000 to 1:10,000 (final).

Mucus
Collect mucus as described in the TECO® Mucus Collection Set TE1034. For assay, add 500 µl **Extraction Buffer** (TECO® Mucus Collection Set, TE1034) to the swab 15-30 min and vortex. For more determinations (e.g. total protein, Cortisol etc.) the swab should be removed from each vial and discarded before Hyaluronic acid (HA) measurement. Before pipetting samples into wells repeat vortexing the sample. In most studies, the samples should be used without any further dilution.

Correction of HA results by protein correction
Independently from the assay procedure, various factors may influence the final amount of biological sample added to the Hyaluronic Acid ELISA (e.g. total amount of blood collected into the prefilled sample tubes; effectiveness of homogenization; amount of mucus on the swab etc.). In order to obtain the comparable analytical results, all samples may be corrected by the protein concentration by using in parallel a colorimetric protein determination. The VTG Dilution Buffer and the Extraction Buffer are protein free and may be used as standard buffers and for sample dilution in the protein assay. The sample dilution of the protein assay may differ from the optimal sample dilution in the hyaluronic acid assay.

Result analysis

Please note that the samples might not be diluted with the same dilution factor as the standard curve (1:50). To correct this difference, the following formula can be used:

$$\frac{\text{"Final dilution factor" of samples}}{\mathbf{50}\ (\text{dilution factor of standard curve})} = \text{"Calculation dilution factor"}$$

The “calculation dilution factor” should be multiplied with the measured HA concentration to obtain the HA concentration.

This HA concentration can then be related to the protein concentration of each sample.