biosense®
LABORATORIES

SEMI-QUANTITATIVE BIOMARKERS ELISA KIT COMPONENTS FOR FISH SAMPLES

PROD. NO.: B00400402 / B00400404

CONTENTS

A. INTRODUCTION

This kit contains a set of Enzyme-Linked Immunosorbent Assay (ELISA) reagents to be used for semi-quantitative detection of biomarkers such as Vitellogenin (Vtg), Cytochrome P450 1A (CYP1A), *Zona radiata* protein (Zrp) and Metallothionein (MT) in samples from fish.
The assay is based on detection of the biomarker using a suitable monoclonal or polyclonal antibody in an indirect antibody capture ELISA format (Figure 1). As a positive control, purified protein (for example Vtg) or a sample that is known to be positive, should be used.

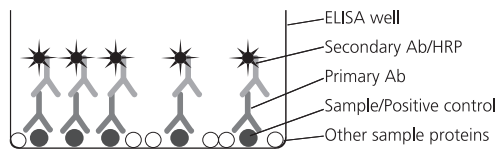

FIGURE 1:
ASSAY FORMAT FOR THE SEMI-QUANTITATIVE ELISA

B. SAFETY INSTRUCTIONS

For research use only. Not for human use or drug use. Not for clinical diagnostic use. These reagents contain sodium azide as preservative. Do not use internally or externally in humans or animals. The kit contains OPD (*o*-phenylenediamine) tablets. Since OPD is toxic and may be carcinogenic, contact should be avoided and gloves and suitable protective clothing should be used when handling tablets or solutions made from the tablets (see Material Safety Data Sheet [MSDS] for the OPD tablets for more details about proper handling and waste disposal). As all chemicals should be considered potentially hazardous, it is advisable to wear suitable protective clothing during handling of this kit. Avoid contact with skin and eyes.

C. STORAGE AND STABILITY

Store the kit at 2-8°C upon arrival. Do not freeze. See expiry date on the kit box for stability of the kit.

D. WARRANTY AND LIMITATION OF REMEDY

Biosense Laboratories AS (hereafter: Biosense) makes no warranty of any kind, expressed or implied, including, but not limited to, the warranties of fitness for a particular purpose and merchantability, which extends beyond the description of the chemicals on the face hereof, except that the material will meet our specifications at the time of delivery.
Buyer's exclusive remedy and Biosense´s sole liability hereunder shall be limited to refund of the purchase price of, or at Biosense´s option, the replacement of, all material that does not meet our specifications. Biosense shall not be liable otherwise or for incidental or consequential damages, including, but not limited to, the costs of handling.
Said refund or replacement is conditioned on Buyer giving written notice to Biosense within thirty (30) days after arrival of the material at its destination, and Buyer treating the material as outlined in the product data sheet and/or kit insert after arrival. Failure of Buyer to give said notice within said thirty (30) days, or failure of Buyer in treating the material as outlined in the product data sheet and/or kit insert shall constitute a waiver by Buyer of all claims hereunder with respect to said material.
The responsibility of all patent considerations in the use of our products rests solely with the user.

E. KIT CONTENTS

		1-plate kit	5-plate kit
A)	96 well microplates	1	5
B)	Coating buffer capsules	1	2
C)	Phosphate buffered saline (PBS) tablets	1	3
D)	PBS/Tween tablets	1	3
E)	Bovine serum albumin (BSA)	0.5 g	2.5 g
F)	Primary antibody, *optional*	*100 µl*	*500 µl*
G)	Secondary antibody, concentrated 2000x Horseradish peroxidase (HRP) conjugate	1 vial	1 vial
H)	OPD-peroxidase substrate, tablet sets	1 set	3 sets
I)	Positive control, Vtg standard*, optional* Purified, lyophilised Vtg	*1 vial*	*2 vials*

F. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

In addition to the reagents supplied with the kit, the following reagents and equipment are required and/or recommended to perform the assay:

- 2M H_2SO_4 (stop solution)
- Incubator at 37°C
- Microplate reader (wavelength 492 nm)
- Pipettes with disposable tips (5-1000 µl)
- Multi-channel or stepper pipette with disposable tips (50 and 100 µl)
- Test tubes (1-50 ml)
- Microplate washing device (a manual or automatic plate washer is recommended, but a squeeze bottle or a multichannel/stepper pipette can also be used)
- Vortexer
- Crushed ice

G. IMPORTANT NOTES

1. Read the complete procedure before starting the assay.
2. The assay described in this protocol is a *semi-quantitative* assay, and it is *NOT* suitable for measuring absolute amounts of the biomarker in question. Generally, the level of the biomarker is reflected in the absorbance values obtained in the ELISA; the higher the absorbance value, the higher the level of the biomarker in the sample. Please note that there is no simple, direct correlation between absorbance values and the biomarker concentration. Therefore, samples that are to be directly compared should be run in the same assay.
3. The degree of cross-reactivity between different fish species will depend on the properties of the primary antibodies used. The signal (absorbance) obtained with samples from different fish species are therefore not directly comparable.
4. The optimal sample and antibody dilutions should be determined empirically. The dilution factors recommended in this protocol are examples only, and are representative for antibodies obtained from Biosense Laboratories AS.
5. After blocking and after each wash, empty the wells by inverting the plate over a sink and then tapping it against a pile of paper towels until no fluid is left in the wells.
6. In order to obtain reliable results, several common sources of error should be avoided. Important factors to increase reliability are:
   - Careful and precise pipetting at every step in the assay. Reverse pipetting of the Blocking/Dilution buffer is recommended to increase reliability.
   - Addition of sample and positive control dilutions to the plate in triplicates, instead of duplicates, will increase reliability.
   - Avoid shaking and excess foaming when preparing dilutions. Using a vortexer is recommended.

H. PREPARATION OF BUFFERS/REAGENTS

1. Coating buffer (50 mM carbonate-bicarbonate, pH 9.6):
   Open one coating buffer capsule (vial B) and dissolve the content in 100 ml distilled water.
   Store at 2-4°C (stable for at least two months).
2. PBS (Phosphate buffered saline, pH 7.3):
   Dissolve one buffer tablet (plastic bag C) per 100 ml distilled water.
   Store at 2-4°C (stable for at least one month).
3. Washing buffer (PBS, 0.05% Tween-20):
   Dissolve one buffer tablet (bag D) in 1000 ml distilled water. Store at 2-8°C (stable for at least one month).
4. Blocking/Dilution buffer (1% BSA in PBS):
   Dissolve 0.5 g BSA (vial E) in 50 ml PBS for each plate used in the assay.
   Store at 2-4°C (stable for 2-3 days).
5. Substrate solution (*prepare just prior to use!*):
   Dissolve one Urea Hydrogen Peroxide tablet in 20 ml distilled water (dissolves slowly, 10-15 minutes with gentle shaking). Then add one OPD tablet and let it dissolve. For best results the substrate solution should be used within 30 minutes.
6. Positive control, purified Vtg (vial I, if supplied in the kit): Dissolve the lyophilised Vtg in 100 µl cold PBS to make a stock solution. Make suitable aliquots and store at -20°C, if not used immediately. Avoid repeated freezing/thawing of Vtg.

I. ASSAY PROCEDURE

Day 1: Coating with samples and positive control
See recommended plate layout in Figure 2.

Frozen samples and positive control should be thawed on ice.

1. Dilute samples in Coating buffer:
   Please note: *The optimum coating concentration must be determined for each assay, but these dilutions/concentrations can be generally recommended:*

Liver microsomes:	10 µg/ml
Post-mitochondrial supernatants (PMS):	40 µg/ml
Plasma samples:	1:1000
Purified Vtg:	1-5 µg/ml
Other purified proteins:	5-10 µg/ml

2. Dilute the positive control in coating buffer to reach desired concentration.
   Example: *Dilute Vtg stock solution to 5 µg/ml.*
3. Add 100 µl coating buffer to each of two wells (duplicate analysis) or three wells (triplicate analysis). These wells will be used to determine the Non-Specific Background signal (NSB).
4. Add 100 µl diluted positive control to each of two or three wells.
5. For each sample, add 100 µl to each of two or three wells.
6. Incubate at 4 °C overnight
   Please note: *Coating for 1 hour at room temperature or 37ºC is possible, but this may alter the sensitivity of the assay depending on the nature of the sample.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	NSB	Pos con.	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
B	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
C	P11	P12	P13	P14	etc.							
D	↓	↓	↓	↓	↓							
E												
F												
G												
H												

FIGURE 2:
RECOMMENDED PLATE LAYOUT
NSB= NON-SPECIFIC BINDING WELLS
POS.CON.= POSITIVE CONTROL
P1, P2, P3 ETC.= SAMPLES

Day 2:
Blocking the wells
7. Wash the wells 3 times with 300 µl Washing buffer per well (see note G5).
8. Add 200 µl of the Blocking/Dilution buffer to each well.
   Incubate at room temperature for 30-60 min.

Incubation with Primary antibody
Please note: *See antibody Specification sheet for recommended dilutions of Primary antibodies from Biosense Laboratories AS.*
9. Empty the wells (see note G5).
10. Dilute the Primary antibody (vial F, if supplied in the kit) in Blocking/Dilution buffer.
    Add 100 µl of the antibody solution to each well.
    Incubate at 37 °C for 1 hour.
    Please note: *Incubation with the Primary antibody may be performed for 2 hours at 37 °C or at 4°C overnight. Depending on the Primary antibody, this may alter the sensitivity of the assay.*

Incubation with Secondary antibody
11. Wash the wells 3 times with 300 µl Washing buffer per well (see note G5).
12. Dilute the Secondary antibody (vial G) 1:2000 by adding 6 µl antibody to 12 ml Blocking/Dilution buffer for each plate run in the assay.
    Add 100 µl of the antibody solution to each well.
    Incubate at room temperature for 1 hour.
    Please note: *Increasing the incubation time of the Secondary antibody to 2 hours or incubating at 37ºC may increase assay sensitivity.*

Development of the plate
Please note: *The Substrate solution should be prepared just before proceeding to the next step.*
13. Wash 5 times with 300 µl Washing buffer per well (see note G5).
14. Add 100 µl substrate solution to each well. Incubate at room temperature for 15 min
    Please note: *If the reaction is weak, the incubation time may be extended up to 30 min.*
15. Stop the reaction by adding 50 µl 2M H_2SO_4 to each well.
16. Read the absorbance at 492 nm.

J. PREPARATION OF DATA

Subtraction of NSB absorbance values:
On each plate, calculate the mean of the absorbance values of the NSB wells and subtract this value from the absorbance values of all other wells on the same plate. This gives the NSB-corrected absorbance values for standard and sample dilutions.

NOTES

NOTES

K. QUICK GUIDE

1. Thaw samples on ice.
2. Prepare dilutions of samples and positive control in Coating buffer.
   Add Coating buffer to NSB wells.
   Add diluted samples and positive control to plate.
   Incubate overnight at 4ºC.
4. Wash the plates 3 times with 300 µl Washing buffer per well.
5. Add 200 µl Blocking/Dilution buffer to all wells.
   Incubate 30-60 min at room temperature
6. Empty the plate.
   Add 100 µl diluted Primary antibody to all wells.
   Incubate at 37°C for 1 hour.
7. Wash the plates 3 times with 300 µl Washing buffer per well.
   Add 100 µl diluted Secondary antibody to all wells.
   Incubate at room temperature for 1 hour.
7. Wash the plates 5 times with 300 µl Washing buffer per well.
   Add 100 µl Substrate solution to all wells.
   Incubate in the dark at room temperature for 15 minutes.
8. Add 50 µl 2M H_2SO_4 to all wells to stop the reaction.
   Read the absorbance at 492 nm.

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