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– XEMA's OEM partner for Food testing ELISA kits in USA

Preliminary Performance Evaluation of the Fish EIA

1.0 Overview

The goal was to evaluate the performance of the Fish EIA using a panel of extracted fish and seafood samples.

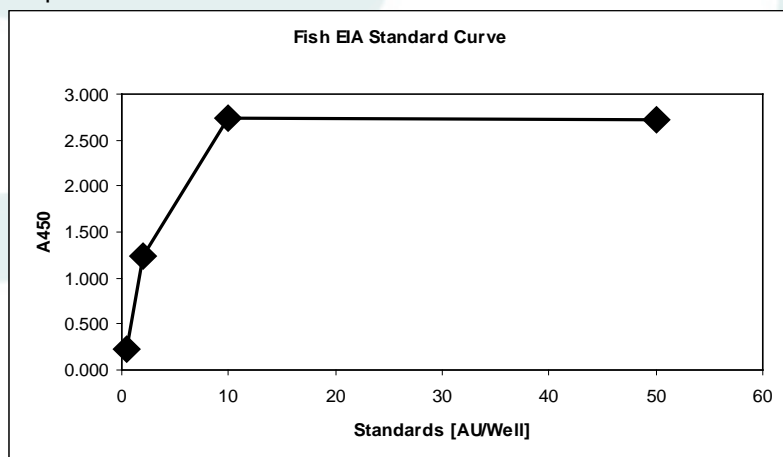
2.0 Methods

Fish and seafood samples were extracted using a proprietary method and the protein concentration of each sample was estimated by using A280 ($\epsilon=1.4$). The extracted samples were diluted if necessary and run in parallel with the kit standards per the ELISA protocol.

3.0 Results

The results of the kit standard curve are shown in Graph 1. The data demonstrated that the standard curve absorbance was maximal at Standard 3 and would be most effective in estimating concentrations in the range below Standard 3. Based on this, we added 3 additional concentrations below Standard 3 to improve the estimation of sample concentration within this range (Graph 2). A linear regression was performed and yielded a linearity of $r=0.996$ (Graph 2). We ran known concentrations of 25 fish and seafood samples at various dilutions within the range of the standard curve in Graph 2 and the standard curve in parallel (Table 1). The Fish EIA detected every fish sample. Since we knew the concentration of each extract, we standardized the absorbance readings by dividing the extract concentration (ng/ml) by the absorbance to yield ng of extract per AU (ng/AU, Table 1). This enables one to rank the sample in order of reactivity. Based on this, the most reactive fish sample was Snapper at 0.444 ng/AU and the least reactive fish was Mackerel at 20825.853 ng/AU. Also, the seafood samples showed low reactivity with Crab at 12684.366 ng/AU and Lobster at 1.25E6 ng/AU.

Graph 1. Fish EIA Standard Curve



Graph 2. Fish EIA Adjusted Standard Curve

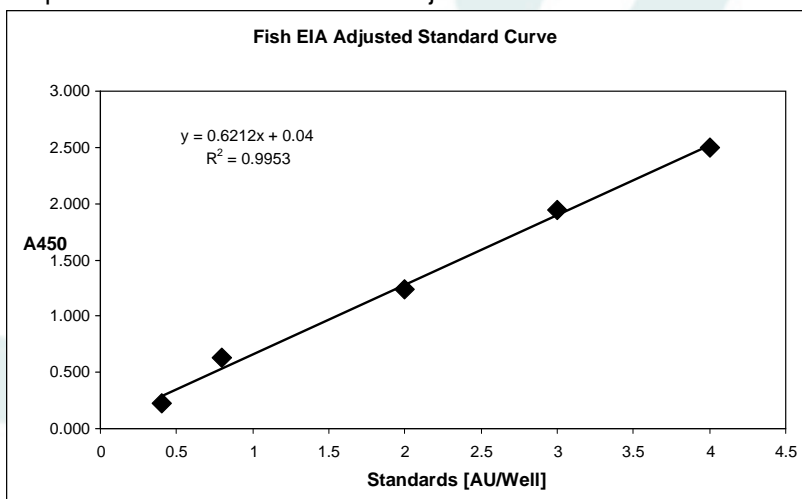


Table 1. Reactivity of Extracted Fish and Seafood Samples

Antigen	Absorbance A ₄₅₀ -BKG	Protein [ug/ml]	Protein [ng/well]	Protein [ng/AU]
Anchovy	0.722	18.856	942.8	1305.817
Anglerfish	0.642	0.17616	8.8	13.707
Catfish	0.459	0.075	3.8	8.279
Cod	0.623	0.3504	17.5	28.090
Flounder	1.282	0.05616	2.8	2.184
Grouper	0.440	0.133	6.7	15.227
Hake	0.928	0.0752	3.8	4.095
Halibut	1.066	0.09568	4.8	4.503
Mackerel	0.557	232.0	11600.0	20825.853
Orange Roughy	0.924	0.02896	1.4	1.515
Pike	0.392	0.2392	12.0	30.612
Salmon	0.368	0.1192	6.0	16.304
Sardine	0.376	2.1056	105.3	280.053
Sea Bass	0.400	0.08704	4.4	11.000
Sea Bream	0.456	0.16256	8.1	17.763
Snapper	1.352	0.0121	0.6	0.444
Sole	1.391	0.0448	2.2	1.582
Swordfish	1.521	0.8416	42.1	27.679
Trout	1.047	2.6112	130.6	124.737
Tuna	1.125	0.1644	8.2	7.289
Turbot	0.526	0.06832	3.4	6.464
Crab	0.339	86.0	4300.0	12684.366
Lobster	0.004	100.0	5000.0	1.25E6
Scallops	0.168	119.0	5950.0	35416.667
Shrimp	0.193	398.5	19925.0	103.2E3

4.0 Conclusions

The Fish EIA is an easy kit to run and shows broad reactivity with all fish samples, while at the same time, shows low cross reactivity with un-related seafood samples. The sensitivity is in the nano-picogram range. Because this kit is so sensitive, it may be useful to expand the standards in the lower range. Overall, this EIA would be very useful to detect contamination of food stuffs with fish-antigens. Foods found to be contaminated with fish antigens could be identified prior to ingestion and unintended allergic reactions to fish antigens would be prevented.