行政院國家科學委員會專題研究計畫 期末報告

以新穎的過氧化醯促進劑開發靈敏之冷光酵素免疫分析法來分析食品中的毒素(第 2 年)

計 畫 類 別 ： 個別型
計 畫 編 號 ： NSC 100-2923-B-040-001-MY2
執 行 期 間 ： 101 年 08 月 01 日至 102 年 07 月 31 日
執 行 單 位 ： 中山醫學大學生物醫學科學學系（所）

計 畫 主 持 人 ： 余豐益
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公 開 資 訊 ： 本計畫涉及專利或其他智慧財產權，1 年後可公開查詢

中 華 民 國 102 年 11 月 05 日

中 文 摘 要 ： 黃麴毒素(aflatoxin B1/M1)與黑海棉酸(okadaic acid)主要由黴菌 Aspergillus 屬與藻類等常產
生的黴菌毒素與藻類毒素，此兩類毒素泛存於食品與飼料中，食用遭受污染的食品
會導致人類許多疾病及癌症的生成。由於此類毒素對於人類及動物體健康有高度的威脅，
因此利用實驗室已開發的抗體及建立免疫分析法來分析調查食品的黃麴毒素與黑海棉酸的污染是一
重要的課題。此一計畫利用過氧化氫來氧化 phenothiazines 調控辣根過氧化酵素(horseradish
peroxidase)的活性，以增強冷光的強度及長時間的穩定度分別開發黃麴毒素(aflatoxin B1)與黑海
棉酸之冷光酵素免疫分析法來增進分析靈敏度，使得偵測極限更低。在黃麴毒素方面冷光酵素免疫分
析法偵測限制為 0.0015 ng/ml,工作濃度範圍為 0.003~0.03 ng/ml； 在黑海棉酸方面，偵測限制為 0.01
ng/ml,工作濃度範圍為 0.03~0.2 ng/ml。

中文關鍵詞： 黃麴毒素； 黑海棉酸； 冷光酵素免疫分析法

英 文 摘 要 ： Aflatoxin B1/M1 and okadaic acid are natural carcinogenic toxinswhich produced by funi and algae,respectively, They are commonly contaminated in food, milk and feed which cause toxic effects and cancer in human andanimal. Development of enzyme-linked immunosorbent assay (ELISA) for Aflatoxin B1/M1 and okadaic acid are important issue in food safety. Therefore, in order to improve the sensitivity of ELISA method, chemiluminescent competitive direct enzyme-linked immunosorbent assay (CL-cdELISA) for
determination of AFB1/M1 and okadaic acid need to be developed. An ultrasensitive CL-ELISA was developed by using the oxidation of phenothiazines by hydrogen peroxide in the presence of horseradish peroxidase. The values of the detection limit value and dynamic working range of CL-ELISA of AFB1 were 0.0015 ng/mL and 0.003 – 0.03 ng/mL； In the okadaic acid, The values of the detection limit value and dynamic working range of CL-ELISA of okadaic acid were 0.01 ng/mL and 0.03 – 0.2 ng/mL.

英文關鍵詞： aflatoxin B1； okadaic acid； chemiluminescent

competitive direct enzyme-linked immunosorbent assay
(CL-cdELISA

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

以新穎的過氧化酶促進劑開發靈敏之冷光酵素免疫分析法來分析食品中的毒素

計畫類別：■個別型計畫　□整合型計畫
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計畫主持人：余豐益
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計畫參與人員：洪駿澤, 呂權蓁

本計畫除繳交成果報告外，另含下列出國報告，共 _ 份：
□移地研究心得報告
□出席國際學術會議心得報告
□國際合作研究計畫國外研究報告

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中　華　民　國 102 年 10 月 31 日

行政院國家科學委員會專題研究計畫期末報告

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主持人：余豐益　　中山醫學大學生物醫學科學系

中文摘要:

黃麴毒素(aflatoxin B1/M1)與黑海棉酸(okadaic acid)主要由黴菌*Aspergillus* 屬與藻類等常產生的黴菌毒素與藻類毒素，此兩類毒素泛存於食品與飼料中，食用遭受污染的食品會導致人類許多疾病及癌症的生成。由於此類毒素對於人類及動物體健康有高度的威脅，因此利用實驗室已開發的抗體及建立免疫分析法來分析調查食品的黃麴毒素與黑海棉酸的污染是一重要的課題。此一計畫利用過氧化氫來氧化phenothiazines調控辣根過氧化酵素(horseradish peroxidase)的活性，以增強冷光的強度及長時間的穩定度分別開發黃麴毒素(aflatoxin B1)與黑海棉酸之冷光酵素免疫分析法來增進分析靈敏度，使得偵測極限更低。在黃麴毒素方面冷光酵素免疫分析法偵測限制為0.0015 ng/ml,工作濃度範圍為0.003~0.03 ng/ml; 在黑海棉酸方面，偵測限制為0.01 ng/ml,工作濃度範圍為0.03~0.2 ng/ml。

英文摘要:

Aflatoxin B1/M1 and okadaic acid are natural carcinogenic toxinswhich produced by funi and algae, respectively, They are commonly contaminated in food, milk and feed which cause toxic effects and cancer in human andanimal. Development of enzyme-linked immunosorbent assay (ELISA) for Aflatoxin B1/M1 and okadaic acid are important issue in food safety. Therefore, in order to improve the sensitivity of ELISA method, chemiluminescent competitive direct enzyme-linked immunosorbent assay (CL-cdELISA) for determination of AFB1/M1 and okadaic acid need to be developed. An ultrasensitive CL-ELISA was developed by using the oxidation of phenothiazines by hydrogen peroxide in the presence of horseradish peroxidase. The values of the detection limit value and dynamic working range of CL-ELISA of AFB1 were 0.0015 ng/mL and 0.003–0.03 ng/mL; In the okadaic acid, The values of the detection limit value and dynamic working range of CL-ELISA of okadaic acid were 0.01 ng/mL and 0.03–0.2 ng/mL

【I】前言與目的:

黃麴毒素(aflatoxins)是一族主要由 *Aspergillus* 屬黴菌所產生的二級代謝產物，此類毒素可能在採收前後或採收後儲藏運送的過程中污染穀物與穀類製品，例如花生及玉米 (IARC, 1993)，根據 FAO (Food and Agriculture Organization) 統計結果指出，世界上有約 25%的農產品遭受黴菌毒素的污染，所以導致農業經濟上嚴重的損失。黃麴毒素共分四大種類，其中以黃麴毒素 B1 最癌性，而且黃麴毒素結構非常穩定，不會因為加熱或是食品加工而失去毒性。目前國際癌症研究協會 (International Agency for Research on Cancer) 將黃麴毒素 B1 評定為第 1 級的人類致癌物質。目前許多國家對於食物中黃麴毒素 B1 的含量設有 20 ppb 的含量限制。台灣食品藥物管理局在 2009 年抽檢花生製品發現，黃麴毒素 B1 污染污染嚴重者高達 203 ppb 大約高出限制含量 15 ppb 的約 14 倍。2004 年在肯亞也因食用受到黃麴毒素污染的玉米而造成 317 人中毒，最後導致 125 人因為肝癌而死亡的案例 (Eduardo et al., 2005)；此外台灣與美國最近常有報導指出犬類食用遭受污染的飼料而導致腎衰竭與肝臟傷害的事件，其主要污染源經證實為黃麴毒素 B1。最新研究指出黃麴毒素 M1 常常污染牛奶與奶製品並可能與肝癌的盛行具有高相關性 (Peng, 2009; Prandini, 2009)。

軟海綿酸 (Okadaic acid, OA) 是一種常見於貝類中的藻類毒素，是由 *Dinophysis Prorocentrum* 這兩屬渦鞭毛藻所產生的次級代謝物，經由濾食性貝類食用而累積於體中，特別是二枚貝，像是紫貽貝與海扇貝等等。研究顯示 OA 及其經生物代謝後之衍生物 dinophysistoxins (DTXs) 為引起下痢性貝毒 (diarrhetic shellfish poisoning，DSP) 的主要毒素，在誤食的幾小時內會產生腹瀉、噁心嘔吐與腹痛等症狀並持續三到四天。OA 的分子式為 $C_{44}H_{68}O_{13}$，分子量為 804.5，屬於類脂溶性之長鏈聚醚毒素，對熱穩定但對光較敏感。OA 會抑制蛋白質磷酸酶 (protein phosphatases 1 and 2A) 的作用，使蛋白質磷酸化增加，研究指出 OA 主要影響的器官為腸道，會造成脂質的過氧化 (Guzman and Castro, 1991)。此外，OA 被懷疑會促進消化道腫瘤之生成。歐盟對 OA 的限制含量為 160 ng/g (ppb)，超過此含量就容易造成急性中毒。

二、 材料與方法

2.1 Materials.

Okadaic acid (OA) was purchased from Taiwan Algal Science (Tauyuan, Taiwan). A analytical standard solution of OA (25 μg/mL), bovine serum albumin (BSA), Tween 20, luminol, pristane, dimethyl sulfoxide (DMSO), 1-ethyl-3-(3′-dimethylaminopropyl) carbodimide (EDC), and N-hydroxysuccinimide (NHS) were obtained from Sigma Chemical Co. (St. Louis, MO). Dulbeco Modified Eagle's Medium (DMEM) was obtained from GIBCO Laboratories (Grand Island, NY). Sodium 3-(10′-phenothiazinyl) propane-1-sulfonate (SPTZ) was prepared as described in. 4-Morpholinopyridine (MORPH) was obtained from Aldrich (USA); Tris and H_2O_2 (30%) were from J. T. Baker (USA). Methanol was obtained from Merck (Germany). Black polystyrene plates (MaxiSorb) were obtained from Nunc (Demark).

2.2. Monoclonal antibody specific to OA

The monoclonal antibodies specific to OA (anti-OA-mAb OA) was generated in our laboratory which was from a stable hybridoma cell line, 6B1A3, generated by the fusion of P3/NS1/1-AG4-1 myeloma cells with spleen cells isolated from a BALB/c mouse immunized with OA-γ-globulin. The 6B1A3

mAb belongs to the immunoglobulin G1 (K chain) isotype. For production of the monoclonal antibody, female BALB/c mice (10 weeks old), were injected intraperitoneally with 0.5 mL pristane 7 days before receiving an intraperitoneal injection of $2x10^6$ hybridoma cells suspended in DMEM. Ascites fluid developed 2–3 weeks after the injection of the cells and was collected every other day for 3 days. The ascites fluid was centrifuged at 7000 rpm (5900g) for 5 min to remove the cell debris. The IgG from the cleared ascites fluid was purified by ammonium sulfate precipitation (50% saturation for the final solution) twice and dialyzed against 2 L of phosphate buffered saline (PBS) for 72 h at 4 1C with two changes of buffer and then stored at −70 1C.

2.3. Conjugation of OA and horseradish peroxidase

Conjugation of OA and HRP was carried out by using the EDC/NHS method. Briefly, 0.2 mg of OA in 0.1 mL of DMSO was mixed with 0.6 mg of EDC and 0.4 mg of NHS, and then a HRP solution (0.8 mg of HRP in 0.3 mL of 0.1 M carbonate buffer, pH 9.6) was added. After being stirred at room temperature for 2 h, the OA-HRP conjugates without further purification was dialyzed
against 0.01 M PBS for 72 h and then lyophilized for future use.

2.4. Determination of OA by CL-ELISA

CL-ELISA for determination of OA was carried out using 96-wells black polystyrene plates (MaxiSorb). The diagram of direct competitive CL-ELISA is shown in Fig. 1. The capture antibodies
were coated by adding 100 μL of the solution of the monoclonal anti-OA antibody (dilution 1:5000–1:20,000) in the PBS to each plate well and incubated at 4 C overnight. The plate was then washed by PBST four times using ELx 50 ELISA washer (Bio-Tek instruments, USA) and blocked by adding 170 μL of PBS containing 0.1% BSA for 30 min at 37 1C. The plate was washed four times with PBST. Subsequently, 50 μL of the OA standard in PBS concentrations from 1.0 pg/mL to 2.0 ng/mL or samples simultaneously with50 μl of the OA-HRP conjugate (1:15,000–45000) in PBS were added and incubated at 37 1C for 1 h. After washing the plate as described above, 100 μL of freshly prepared substrate solution (80 mM Tris, pH 8.3, containing 0.17 mM luminol, 2.1 mM SPTZ, 8.75 mM MORP, and 1.75 mM H_2O_2 were added to each well and stirred. Chemiluminescence intensity was monitored after5 min at room temperature on a luminescence reader (FlexStation
3, Molecular Devices, USA).

2.5. CL-ELISA of real shellfish samples

Nineteen shellfish samples purchased from Taiwanese stores were used to determine the OA levels. The preparation of sample was performed in accordance with a protocol described in.
Briefly, each sample (20 g) was homogenized with 80 mL of extraction solvent (100% methanol) and incubated for 30 min with shaking (200 rpm) at 37C. After centrifugation at 14,000g for
10 min the extract was passed through a 0.45 μm syringe filter. The obtained extract was diluted in 5 times with PBS, and then directly subjected to CL-ELISA.

三. 結果(Results)

3.1 Optimization of OA CL-ELISA.

Favorable conditions for the performance of OA CL-ELISA were estimated. Concentrations of the coating anti-OA antibody (anti-OA-mAb) and the conjugate of OA-horseradish peroxidase (OA-HRP) were

varied to optimize the conditions of the chemiluminescent assay for generating a set of calibration curves. All curves had a typical calibration curve obtained in the performance of competitive ELISA (Figure 2). The values of IC_{10}, IC_{50}, working range (IC_{20}-IC_{80}) and a ratio of A_{max} to A_{min} (A_{max}/A_{min}) were selected as the parameters used to calculate the assay efficiency.

Table 1 reveals that increasing the dilution of anti-OA antibody from 1:5000 to 1:20000, significantly decreased the values of IC_{10} and IC_{50}. It subsequently shifted the working range in the area of lower concentrations of OA. The highest A_{max}/A_{min} ratio was obtained at an antibody dilution of 1:10000. Additional dilution of the antibody worsened all analytical parameters of the assay.

Comparing the analytical parameters of the calibration curves obtained at the fixing anti-OA-mAb (1:10000) and a variation of OA-HRP conjugate (1:30000-90000) dilutions revealed that the values of IC_{10}, IC_{50} and IC_{20}-IC_{80} resembled each other, whereas using the conjugate with a dilution of 1:60000 yielded the highest ratio of A_{max}/A_{min}. Therefore, the solutions of anti-OA-mAb and OA-HRP conjugate with a dilution of 1:10000 and 1:60000, respectively were selected as optimal ones for CL-ELISA.

Two coating conditions were compared to optimize the CL-ELISA method. In the first method the monoclonal antibodies were coated at 4 ^{o}C overnight or at 37 ^{o}C for 1 hr to run the CL-ELISA. The obtained results demonstrated that the analytical parameters such as IC_{10}, IC_{50} and IC_{20}-IC_{80} of CL-ELISA was similar (data not shown). At the same time, the CV value in the working range of the assay at coating antibody at 4 ^{o}C overnight was 5-fold lower than that at 37 ^{o}C for 1 hr. Moreover, A_{max}/A_{min} ratio at coating antibody at 4 ^{o}C overnight was higher than that at 37 ^{o}C for 1 hr (Table 2). Therefore, the coating of the monoclonal antibodies at 4 ^{o}C overnight was applied in all subsequent CL-ELISA. Following optimization of the CL-ELISA conditions values of IC_{10}, IC_{50} and IC_{20}-IC_{80} were 0.01, 0.07, 0.03-0.2 ng/ml, respectively.

Some immunochemical methods have been developed for OA determinations [21-26]. The most sensitive of these methods was CL-ELISA [23]. However, this work failed to describe the composition of the used substrate solution. The linear range of their CL-ELISA method ranged from 0.08125–20 ng/ml. Therefore, for the CL-ELISA method with use of SPTZ and MORPH developed in this work, its working range was 0.03-0.2 ng/ml, which was more sensitive CL-ELISA method for OA detection..

3.2 Recovery of the Spiked Shellfish Samples.

The ability to evaluate samples using the proposed CL-ELISA method was estimated using OA-spiked shellfish samples. The standard OA toxin was added into OA-free shellfish samples and, then, extracted by methanol. The OA concentrations in the spiked samples were 0.03, 0.1 and 0.2 ng/ml. Table 3 summarizes the results of the determination of OA concentration in shellfish samples. The 2.5-fold dilution of the spiked samples led to a low recovery (54.4%) for the samples with an OA concentration of 0.03 ng/ml and high CV values (15-24%) for all spiked samples (Table 3A). This finding implies that 2.5-fold dilution of the samples does not preventing the matrix effect. Conversely, the recovery values from the 5-fold diluted samples within and between assays were 86.7-111.2% and 95.1-103.7%, respectively (Table 3B). Moreover, the CV within and between assays were 1.4-14% and 4.6-12.3%, respectively. Based on the latter results, we can infer that the proposed CL-ELISA method permits the precise measurement of OA in shellfish samples.

3.3 Analysis of Real Shellfish Samples.

The content of OA in 19 shellfish samples purchased in Taiwanese stores was determined using the proposed CL-ELISA method. All samples were extracted with 100% methanol and diluted with 10 times with 0.01 M PBS before subjecting to the CL-ELISA analysis. Table 4 summarizes the OA concentration in the

studied shellfish samples. Analytical results indicated that 6 of the 19 examined samples were OA-free, whereas the other 13 samples had an OA level ranging from 1.2 to 8.0 ng/g. Since the Maximum Acceptable Level of OA in European Union and Russia is 160 ng/g, we can conclude that all studied samples of shellfish products analyzed with the developed method had at a level below the maximum acceptable level.

四、計畫成果自評

本研究主要目的是利用過氧化氫來氧化 phenothiazines 調控辣根過氧化酵素(horseradish peroxidase)的活性，以增強冷光的強度及長時間的穩定度分別開發黃麴毒素(aflatoxin B1)與黑海棉酸之冷光酵素免疫分析法來改進分析靈敏度。此計劃為一台俄國合計畫，藉由本實驗室已開發成功之黃麴毒素與黑海棉酸之專一性抗體結合莫斯科大學化學系 Prof. Ivan 已有之冷光酵素免疫分析法分別開發黃麴毒素(aflatoxin B1)與黑海棉酸之冷光酵素免疫分析法，並分別分析食品與水產品遭受污染的情形，目前此一合作計畫已在 Talanta(IF=3.498)發表兩篇研究成果(Talanta 2013, 107:25-29; 2013, 116:343-346)，另有一篇文稿投至 Food Chemistry 修改中。此一兩年台俄國合計畫預估可以發表三篇 SCI 論文，成果相當豐碩，在此感謝國科會對此一國合計畫經費支持。

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Table 1. Determination of the optimal concentrations of coating anti-OA-mAb antibody and conjugate OA-HRP in CL-ELISA

Anti-OA-mAb	OA-HRP	A_{max}/A_{min}	IC_{10}, ng/ml	IC_{50}, ng/ml	$IC_{20} – IC_{80}$, ng/ml
1:10000	1:30000	83	0.02	0.1	0.05 – 0.22
1:10000	1:90000	11500	0.02	0.08	0.04 – 0.16
1:5000	1:60000	5100	0.015	0.1	0.04 – 0.29
1:10000	1:60000	25200	0.01	0.07	0.03 – 0.20
1:20000	1:60000	500	0.004	0.03	0.01 – 0.08

Table 2. Analytical parameters of CL-ELISA for OA determination by using different antibody coating conditions

Conditions for coating of the anti-OA-mAb	A_{max}/A_{min}	CV in the working range, %
1 hour, 37°C	25200	15
Overnight, 4°C	139200	2.7

Table 3. Recovery and CV values of OA in spiked shellfish samples using CL-ELISA.

(A) Recovery and CVs at OA determination in the samples with different dilutions

(spiked OA), ng/ml	Dilution of the shellfish sample			
	1 : 2.5		1 : 5	
	Recovery, %	CV, %	Recovery, %	CV, %
0.03	54.4 ± 8.2	15.1	90 ± 7.3	8.2
0.1	72.5 ± 17.4	24.0	86.7 ± 8.4	9.7
0.2	104.9 ± 15.7	15.0	111.2 ± 5.6	5.0

(B) Recovery and CVs of OA for 5-fold diluted samples in within assay and between assay

Expected Conc. (ng/ml)	Within assay (n=3)			Between assay (n=3)		
	Found conc. (ng/ml)	Recovery, %	CV, %	Found conc. (ng/ml)	Recovery, %	CV,%
0.03	0.0270 ± 0.0022;	90.0 ± 7.3	8.2	0.0285 ± 0.0013	95.1 ± 4.4	4.6
	0.0293 ± 0.0004;	97.7 ± 1.3	1.4			
	0.0293 ± 0.0006	97.7 ± 2	2.1			
0.1	0.0867 ± 0.0084;	86.7 ± 8.4	9.7	0.0993 ± 0.0122	99.3 ± 12.2	12.3
	0.1110 ± 0.0052;	111.0 ± 5.2	4.7			
	0.1001 ± 0.0140	100.1 ± 14	14.0			
0.2	0.2224 ± 0.0111;	111.2 ± 5.6	5.0	0.2074 ± 0.0147	103.7 ± 7.4	7.1
	0.2068 ± 0.0097;	103.4 ± 4.9	4.7			
	0.1930 ± 0.0172	96.5 ± 8.6	8.9			

Table 4. Determination of OA in shellfish samples by CL-ELISA

No.of shellfish sample	Concentration of the OA in shellfish sample, ng/g	No shellfish sample	Concentration of the OA in shellfish sample, ng/g
1	1.8	**11**	NF
2	8.0	**12**	NF
3	NF[a]	**13**	NF
4	5.4	**14**	3.8
5	2.5	**15**	NF
6	1.2	**16**	NF
7	3.4	**17**	1.5
8	2.7	**18**	1.3
9	3.6	**19**	1.4
10	2.6		

[a] NF – no found

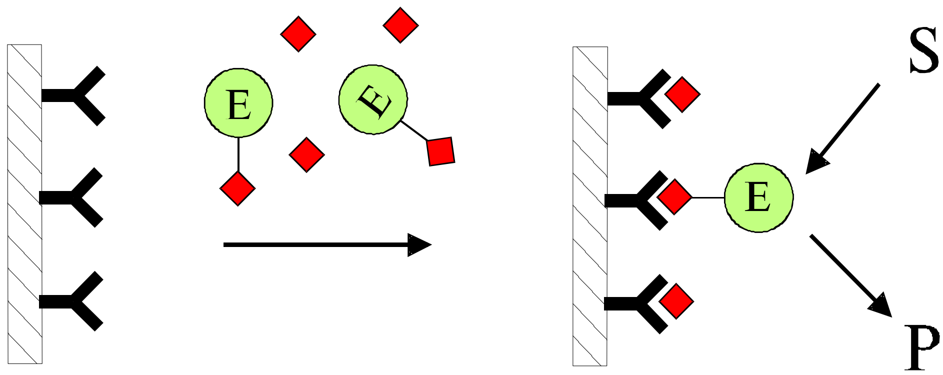

Fig. 1

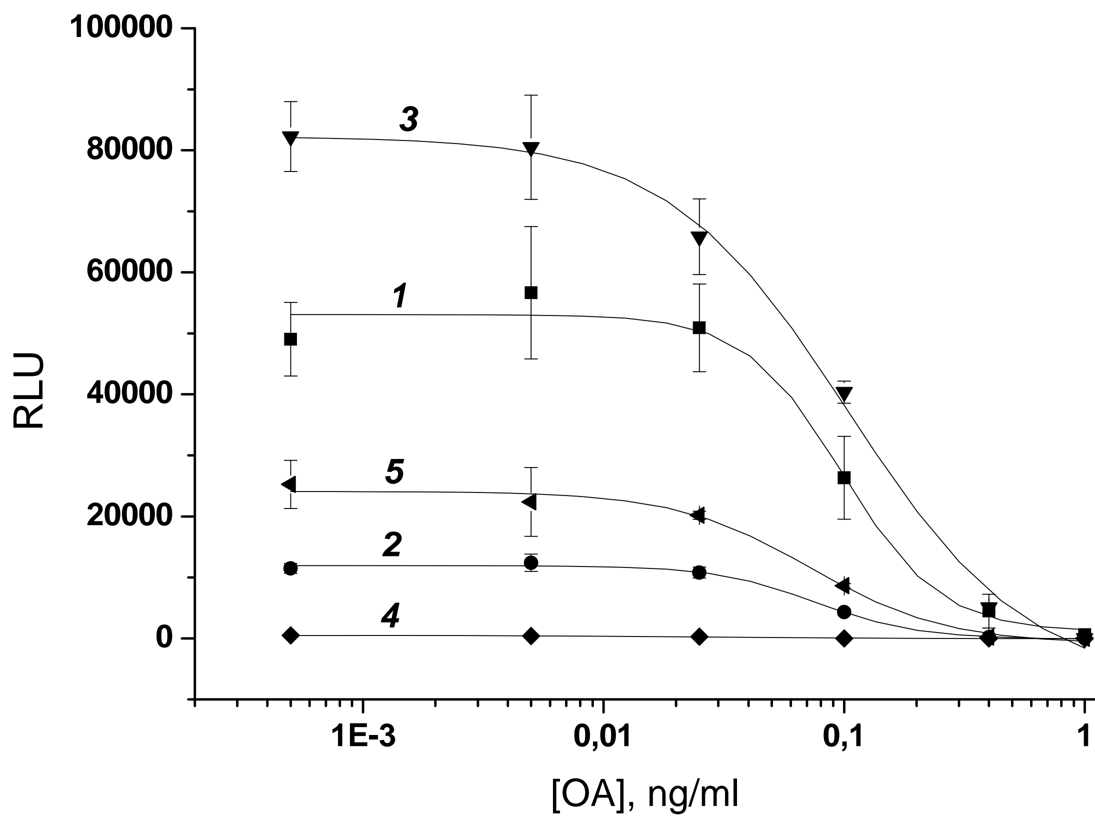

Figure 2.

國科會補助計畫衍生研發成果推廣資料表

日期:2013/11/05

<table>
<tr><td rowspan="3">國科會補助計畫</td><td>計畫名稱: 以新穎的過氧化酶促進劑開發靈敏之冷光酵素免疫分析法來分析食品中的毒素</td></tr>
<tr><td>計畫主持人: 余豐益</td></tr>
<tr><td>計畫編號: 100-2923-B-040-001-MY2　　學門領域: 食品及農化</td></tr>
<tr><td colspan="2">無研發成果推廣資料</td></tr>
</table>

100 年度專題研究計畫研究成果彙整表

計畫主持人：余豐益	計畫編號：100-2923-B-040-001-MY2
計畫名稱：以新穎的過氧化酶促進劑開發靈敏之冷光酵素免疫分析法來分析食品中的毒素	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	3	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	此一兩年期臺俄國合作計畫已在 Talanta(IF=3.498)發表兩篇研究成果(Talanta 2013,107:25-29； 2013,116:343-346)，另有一篇文稿投至 Food Chemistry 修改中。此一兩年台俄國合計畫預估可以 發表三篇 SCI 論文，成果相當豐碩，在此感謝國科會對此一國合計畫經費支持。

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 ■達成目標 □未達成目標（請說明，以 100 字為限） □實驗失敗 □因故實驗中斷 □其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：■已發表 □未發表之文稿 □撰寫中 □無 專利：□已獲得 □申請中 ■無 技轉：□已技轉 □洽談中 ■無 其他：（以 100 字為限）
3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限） 發表三篇論文，並開發靈敏之毒素新穎檢測技術