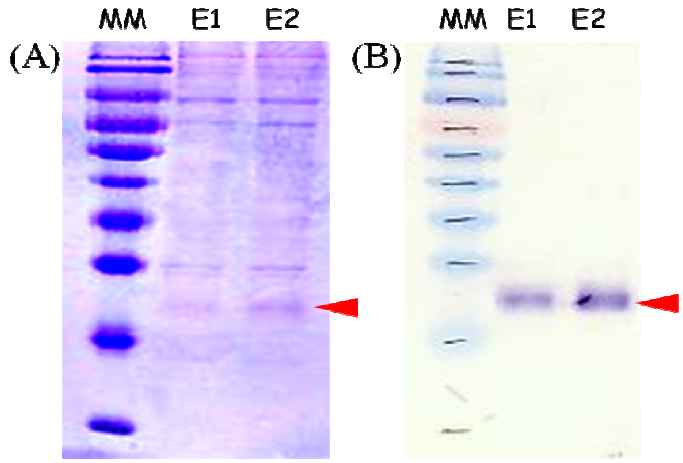
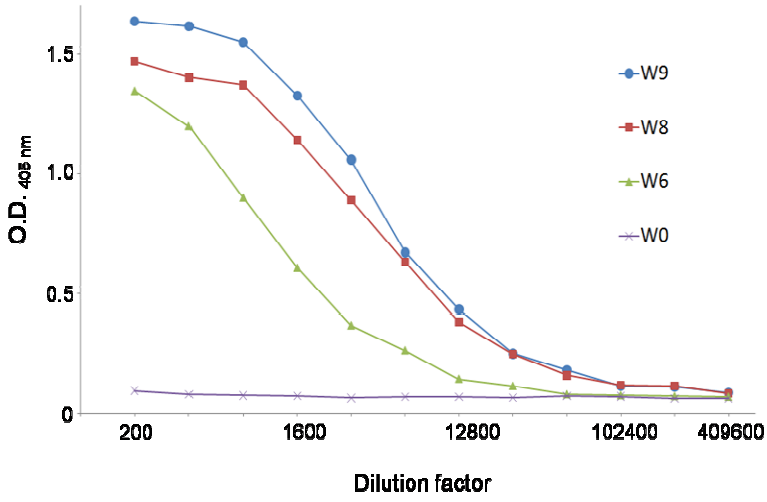
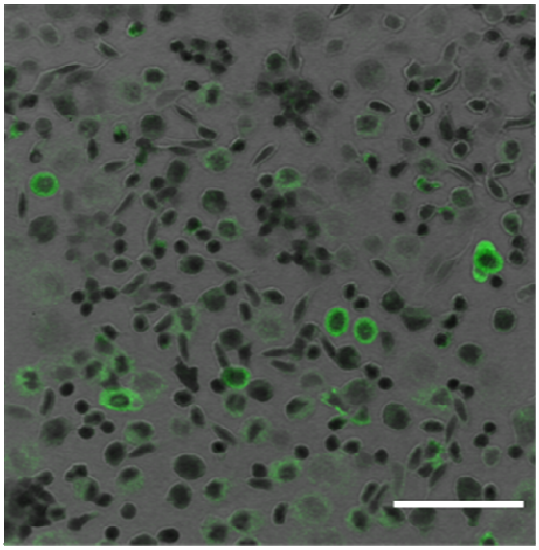
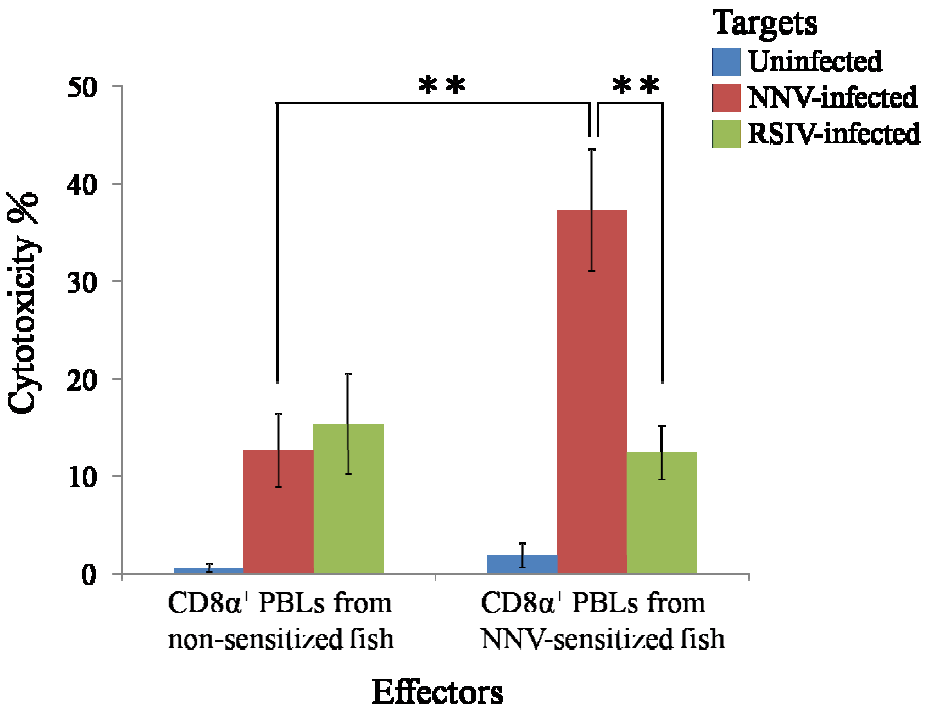
國立臺灣大學技術行銷表

台大案號:09T-100219

產學合作中心聯絡人：駱瑋蓁　　電話： 02-33669948　　e-mail： weichenlou@ntu.edu.tw

產品	1. 點帶石斑魚 細胞毒殺性 T 淋巴球 膜蛋白 CD8α 基因質體 2. 點帶石斑魚 細胞毒殺性 T 淋巴球 膜蛋白 CD8α 重組蛋白 3. 點帶石斑魚 細胞毒殺性 T 淋巴球 膜蛋白 CD8α 兔抗血清
發明人/單位	宋延齡教授　動物學研究所、生命科學系 張允璨　動物學研究所
技術說明	1. 選殖並定性點帶石斑魚 T 淋巴球膜蛋白 CD8α cDNA 2. 利用昆蟲細胞-桿狀病毒表現系統表現出點帶石斑魚 T 淋巴球膜蛋白 CD8α 重組蛋白 3. 免疫大白兔九週後取得點帶石斑魚 T 淋巴球膜蛋白 CD8α 抗血清 4. 抗血清免疫細胞化學染色(Immunocytochemical stain, ICC)可辨識淋巴球次群 5. 抗血清的效價(titer) ELISA 可達 10,000 x， ICC 可達 8,000x
應用範圍	1. CD8α 基因質體：可生物合成 CD8α 重組蛋白 2. CD8α 重組蛋白：可製備 CD8α 抗體、免疫分析中作為競爭者 3. CD8α 抗體：酵素免疫分析(EIA), 西方點墨(Western blot),免疫細胞化學染色(ICC), 免疫組織化學染色(IHC)
產品/技術優勢	1. 商品化魚類抗體很少，商品化魚類白血球或淋巴球抗體更少 2. 提供研究魚類細胞性免疫一個重要的工具，分析魚用疫苗效果的工具 3. 本產品是目前唯一的魚類細胞毒殺性 T 淋巴球膜蛋白 CD8α 抗體
市場潛力	石斑魚是亞洲養殖重要魚種 CD8α 抗體是研究魚類免疫重要的工具
產品/技術 智財權保護方式	以專門技術知識保護

圖片 (已公開之成果可提供圖片)	 圖一、 (A)以 SDS-PAGE 分析經 Ni-NTA agarose 純化的重組點帶石斑魚 CD8α 蛋白產物，箭頭所指處為目標產物，分子量為 24 kDa 的蛋白質色帶。(B)免疫轉漬純化的產物，anti 6×His tag antibody 可辨識到 24 kDa 的色帶(箭頭)。  圖二、利用酵素連結免疫吸附分析法(ELISA)測量石斑魚 CD8α 重組蛋白免疫大白兔 0，6，8，9 週後抗血清的效價. 抗血清作 2 倍連續稀釋.

圖片 (已公開之成果可提供圖片)	 圖三、利用免疫細胞化學染色法染石斑魚周邊白血球的共軛焦顯微圖像。兔抗石斑魚 CD8α 重組蛋白血清作為一抗 (8 週，500x 稀釋)，FITC 標定羊抗兔免疫球蛋白作為二抗(1,000x 稀釋)。圓或卵圓型細胞，中間單型核、高核質比和指環狀細胞質等特徵顯示綠螢光細胞為淋巴球次群。比例尺=25μm  圖四、CD8α+週邊白血球細胞的專一性毒殺。利用 CD8α 抗體分離經過神經壞死病毒病毒暴露一星期的石斑魚 CD8α+週邊白血球細胞，這些白血球細胞會專一性毒殺神經壞死病毒感染、相同基因型鰭細胞，毒殺能力顯著高於被虹彩病毒感染或未感染、相同基因型鰭細胞。顯示 CD8α 抗體所辨識的淋巴球次群是細胞毒殺性 T 淋巴球。

Marketing Abstract of NTU's Invention Disclosure

NTU's docket no:09T-100219

CIAC contact：Lou Wei-Chen Tel：02-33669948 e-mail：weichenlou@ntu.edu.tw

Title	There are three products. 1. CD8α cDNA plasmid. CD8α was the cell surface marker of grouper (*Epinephelus coioides*) cytotoxic T lymphocyte. 2. CD8α recombinant protein 3. Rabbit antiserum against CD8α.
Inventor (s)	Professor Yen-Ling Song, Institute of Zoology and Department of Life Science, College of Life Science Yun-Tsan Chang, Institute of Zoology
Brief Description	1. CD8α cDNA was cloned and characterized from grouper (*Epinephelus coioides*) cytotoxic T lymphocyte membrane protein 2. Recombinant protein of grouper CD8α was biosynthesized using the insect cell-baculovirus expression system. 3. Rabbit antiserum was prepared against CD8α recombinant protein at 8-9 weeks post immunization. 4. The antiserum recognized specifically the subtype of peripheral lymphocytes as confirmed by immunocytochemical stain. 5. The ELISA titer can reach to 10,000 x and the ICC titer to 5,000 x.
Fields of Application	1. The plasmid containing CD8α cDNA can express the CD8α recombinant protein. 2. The CD8α recombinant protein can be used for preparation of the CD8α antibody. The CD8α recombinant protein can also serve as a competitor for CTL binding to MHC class I molecule in the immunoassay. 3. The CD8α antiserum/antibody can be applied in ELISA、Western blot、immunocytochemical (ICC) and immunohistochemical (IHC) stains.
Advantages	1. There are few antibodies available for fish commercially, particularly for fish leucocyte or lymphocyte antibodies. 2. This product will be an important tool for studying the cellular immune responses in fish and useful for evaluating the fish vaccine efficacy. 3. This is the sole product of grouper cytotoxic T lymphocyte CD8α antibody at present.
Market Potential	1. Grouper (*Epinephelus coioides*) is an important fish species for Asian aquaculture. 2. CD8α antibody is an important tool for studying the cellular immune responses in fish.
IP Right(s)	Know-how

Picture

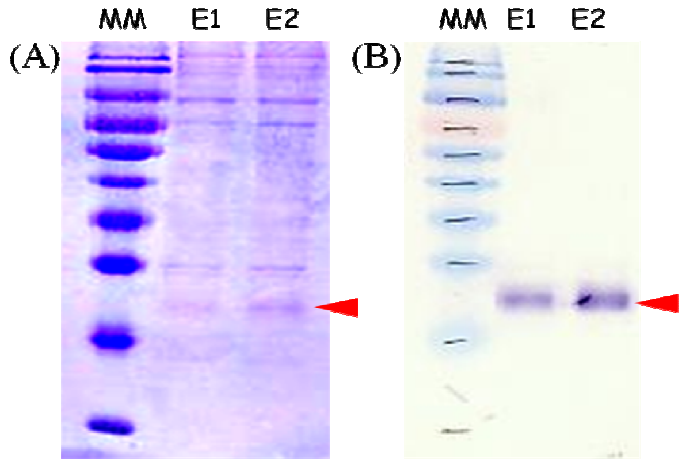


Fig. 1. (A) Reducing SDS-PAGE of recombinant protein stained with Coomassie blue. The arrow head indicates the target product of MW 24 kDa. (B) Western blot of the target product was recognized by anti-6xHis tag antibodies (arrow head). MM: MW marker; E1: Eluted product 1; E2: Eluted product 2.

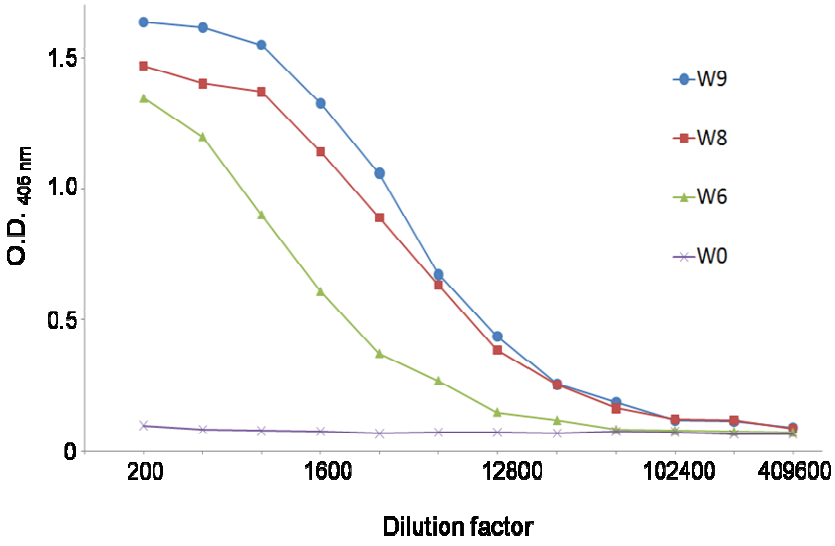


Fig. 2. ELISA titers of rabbit antisera against recombinant grouper CD8α in the weeks post immunization. Rabbit antiserum was 2-fold serially diluted.

Picture

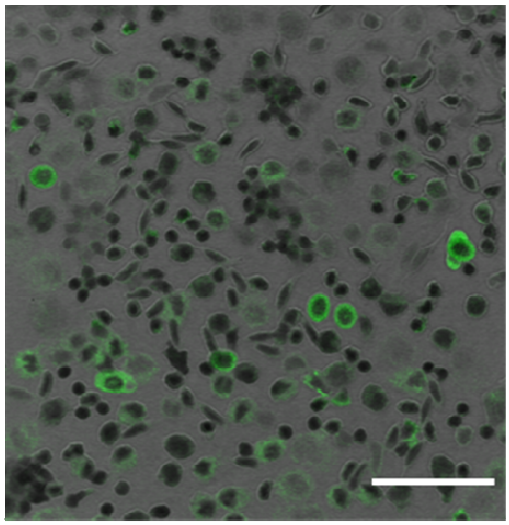


Fig. 3. Confocal microscopy of grouper peripheral blood leucocytes stained with rabbit antiserum against recombinant CD8α (W8, 500x dilution). Rabbit antiserum against grouper CD8α recombinant protein served as the first antibody (W8, 500x). FITC-conjugated goat anti-rabbit Ig served as the secondary antibody. Characteristic round- or ovoid-shaped cells with central monomorphonucleus, high nuclear to cytoplasmic ratio and ring-form cytoplasm indicate the subset lymphocytes. Bar =25 μm.

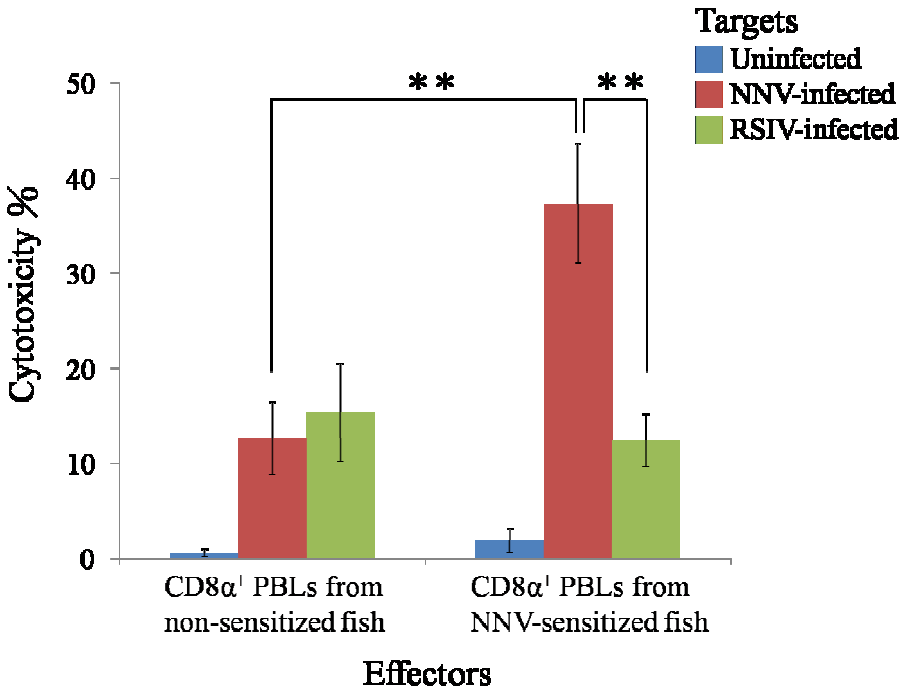


Fig. 4. Specific cytotoxicity of $CD8\alpha^+$ PBL effectors. Harvested from fish at one week post-NNV sensitization, $CD8\alpha^+$ PBL effectors enhanced the cytotoxicity statistically significantly against NNV-infected targets in comparison with RSIV-infected targets. The specific cytotoxicity of $CD8\alpha^+$ PBLs revealed that the lymphocyte subset recognized by CD8α antibodies are the CTLs. Data represent mean±S.D. (n=3).