人源干擾素抗病毒活性
檢測報告

財團法人農業科技研究院
動物科技組
Division of
Animal Technology
Agricultural Technology Research Institute

委託單位: 美商艾諾斯生技股份有限公司
執行單位: 財團法人農業科技研究院
報告編號: AT-002202211-1
報告日期: 2022-11-14

財團法人農業科技研究院
委託測試報告

報告編號：AT-002-202211-1

日期：2022/11/14

頁數：8

以下測試之樣品係由申請廠商所提供並確認資料如下：

申請廠商：美商艾諾斯生技股份有限公司

檢測項目：抗病毒試驗(貓巨噬細胞 Fcwf-4/貓冠狀病毒 FCoV)

樣品名稱：(1) VELDONA® (Human IFN alpha[redacted]

(2) Internal control (feline IFN [redacted]

試驗期間：2022/11/01 ~ 2022/11/04

試驗執行人員：鍾玉東

一、實驗方法：

(一) 樣品配製:

A. stock 濃度為 10^7 U (=10 MU)/ml；[redacted] MIU/mg)用 D-PBS 稀釋；Internal control (10 MU/vial)用檢附的 1 ml 稀釋液回溶。

B. 用細胞培養液進行序列稀釋，stock 先稀釋 100 倍後進行 5 倍序列連續稀釋 9 個濃度，最小濃度 0.0512 U/ml。

C. stock 在步驟 2.前 1 小時配製稀釋後直接使用，餘下 stock 放 4 ℃保存，在步驟 4.稀釋後直接使用。

(二) 實驗步驟:

[抗病毒活性測試]

1、 Fcwf-4 細胞接種 $1*10^4$ cells/well 於 96 孔盤，移至 37 ℃，5 % CO2，培養 24 小時。

2、 細胞控制組和病毒控制組加入不含樣品的培養液；實驗組加入測試樣品 0.1 ml/well 與細胞共培養 24 小時。每組 6 重複。

3、 病毒控制組、實驗組加入 FCoV 100 TCID50/well，於 37 ℃感染作用 2 小時。(細胞控制組不加病毒)

4、 移除含病毒培養液，實驗組加入含測試樣品(濃度同步驟 2)的新培養液 0.1 ml/ well，續培養 42 小時。(細胞控制組和病毒控制組加入不含樣品的培養液)

5、 顯微鏡檢查細胞之病毒感染 CPE (cytopathic effect)

6、 中性紅染色法(neutral red uptake)檢測細胞存活率: 移除培養液，加入 Neutral Red Medium 0.1 ml/well 於培養箱在 37 ℃，5 % CO2 作用 3 小時，移除 Neutral Red Medium，加入 D-PBS 0.15 ml/well 洗滌 2 ~ 3 次，加入 Neutral Red Desorb 0.15 ml/well 震盪 10 分鐘萃取中性紅染劑，用 ELISA reader 偵測波長 540 nm 的吸收值。

7、 以細胞控制組之平均 OD 為 100%存活率，病毒控制組之平均 OD 為 0%存活率，計算實驗組之相對存活率。

$(Mean_{cell\ control} - Mean_{virus\ control})/100$ [Eq. 1]

$(Exp - Mean_{virus\ control})/[Eq.\ 1]*100$ [Eq. 2]

8、 用軟體 GraphPad Prism 8.0 version 計算測試樣品抗病毒活性達到 50%細胞存活之最大效應濃度(EC50)。

Analyzed condition: Nonlinear regression (curve fit) → log(agonist) vs. normalized response—variable slope

Fitting method: Least squares fit

[細胞毒性測試]

1. Fcwf-4 細胞接種 $1*10^4$ cells/ well 於 96 孔盤，移至 37 ℃，5 % CO2，培養 24 小時。
2. 加入測試樣品與細胞共培養 68 小時。(抗病毒試驗時間同步)
3. 移除培養液，加入 MTS/PMS 0.1 ml/well 於培養箱在 37 ℃，5 % CO2 作用 4 小時，用 ELISA reader 偵測波長 490 nm 的吸收值。

二、檢測結果：

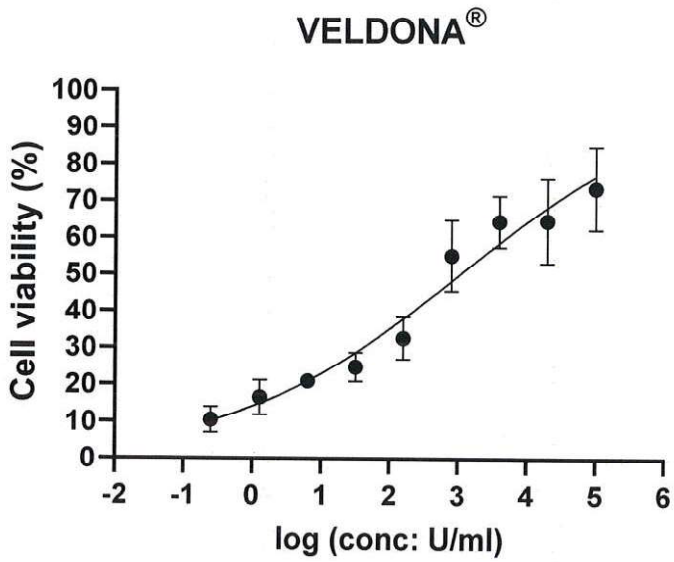
抗病毒活性(Fcwf-4/FCoV)

(一) 中性紅染色 OD 值及細胞存活率(表)

		VELDONA® (Mean ± SD, n=6)		Internal control (Mean ± SD, n=6)	
		OD	(%)	OD	(%)
	Cell control	1.070±0.017	100±2.49	1.078±0.007	100±0.98
	Virus control	0.378±0.023	0±3.38	0.340±0.016	0±2.18
Test sample concentration (U/ml)	100000	[redacted]			
	20000	[redacted]			
	4000	[redacted]			
	800	[redacted]			
	160	[redacted]			
	32	[redacted]			
	6.4	[redacted]			
	1.28	0.492±0.032	16.55±4.66	0.484±0.026	19.44±3.54
	0.25	0.450±0.023	10.45±3.39	0.452±0.027	15.17±3.67
	0.0512	0.466±0.019	12.72±2.80	0.461±0.024	16.35±3.23

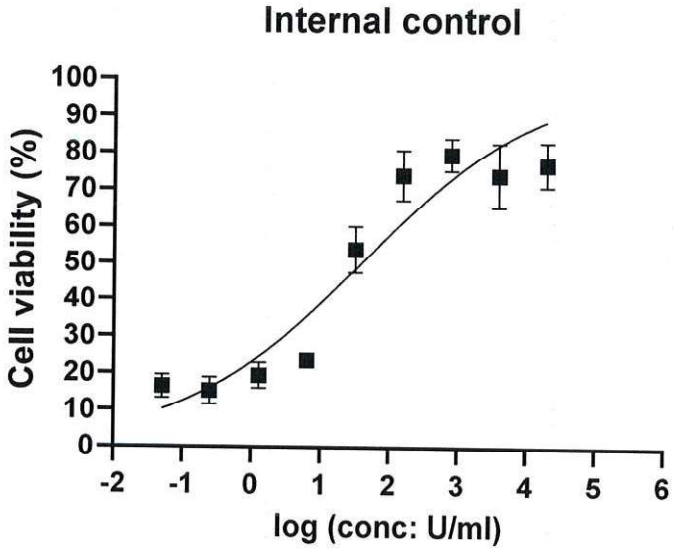
(二) 劑量反應曲線圖(Dose-response curve): (A) VELDONA®，(B) Internal control

(A)



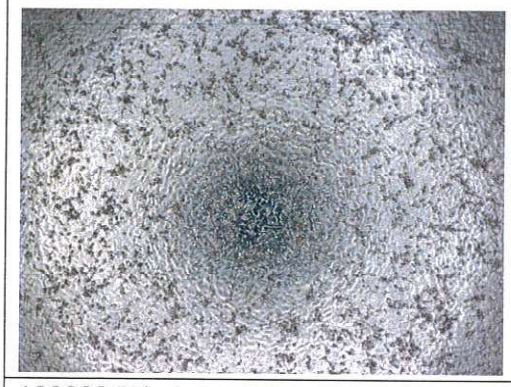
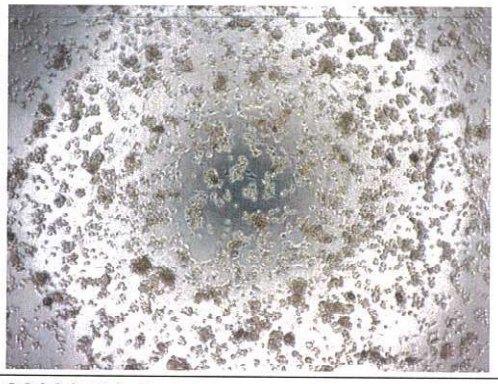
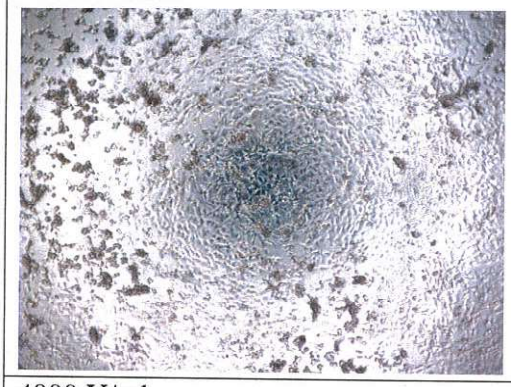
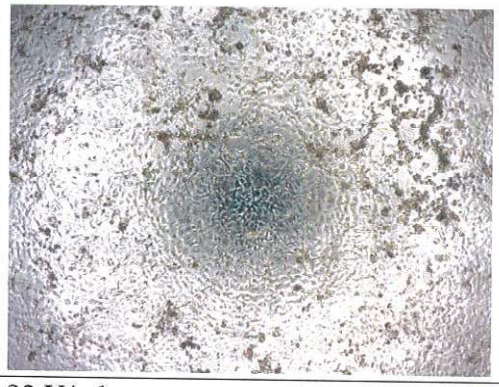
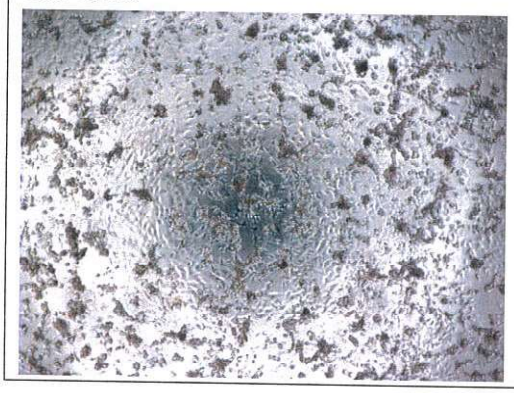
Goodness of fit: R^2= 0.8885
Best fit values:
Hill slope= 0.2599
[redacted]

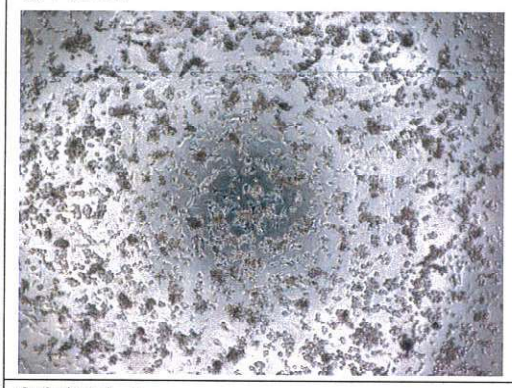
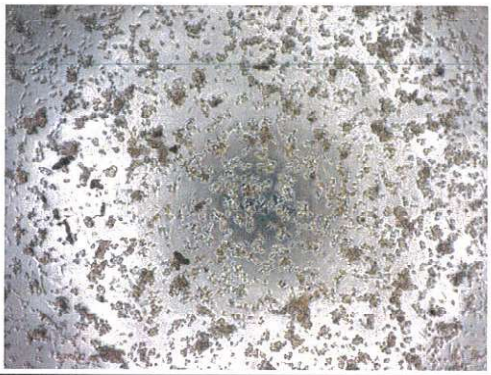
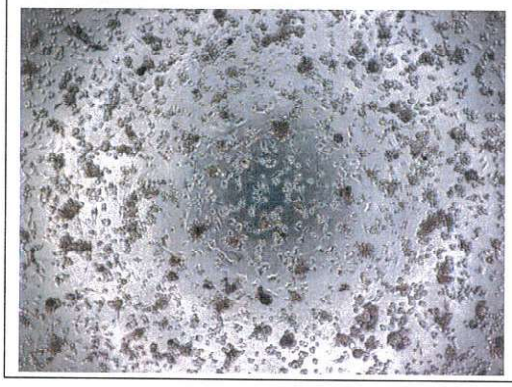
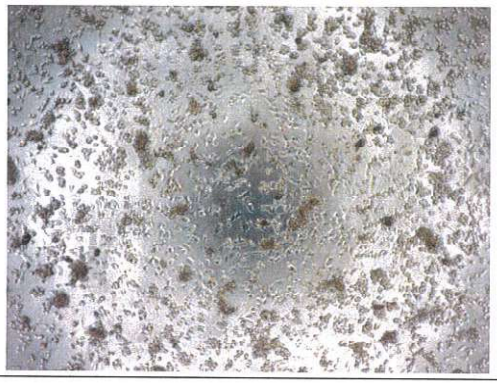
(B)



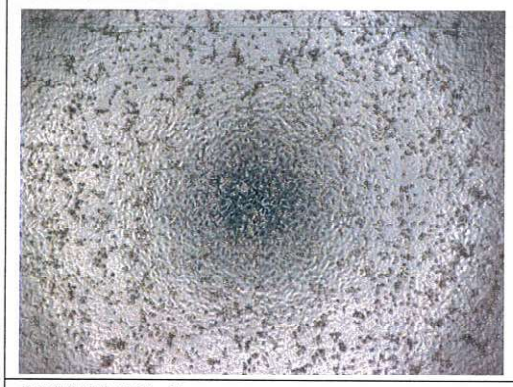
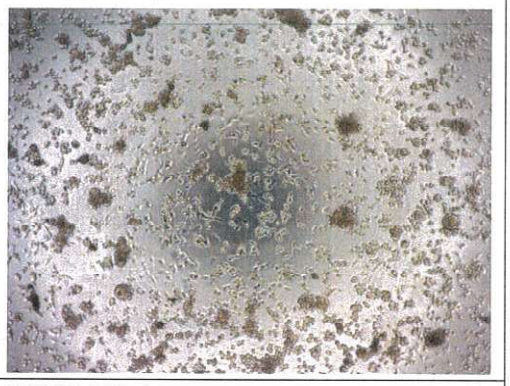
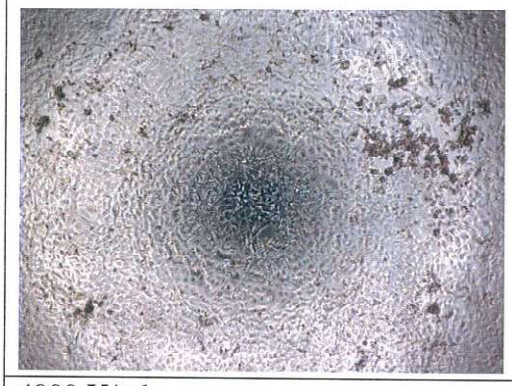
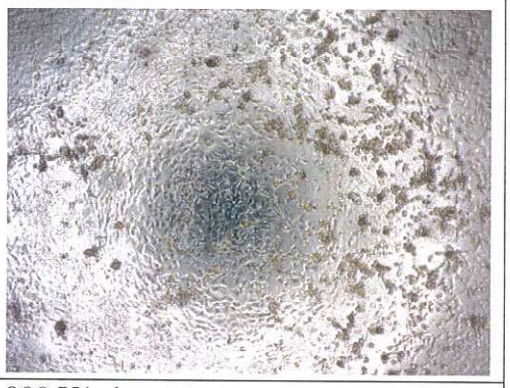
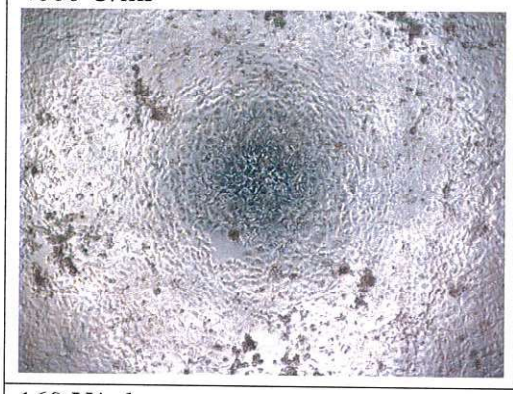
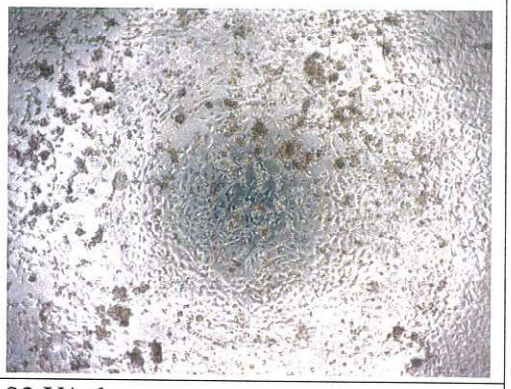
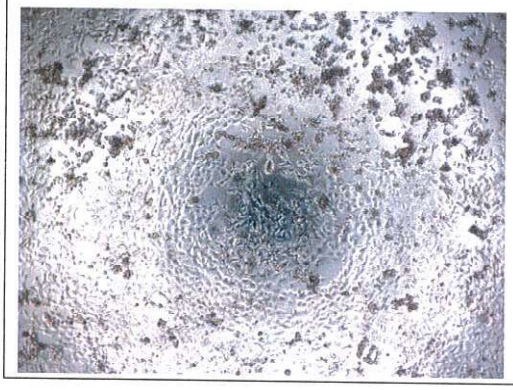
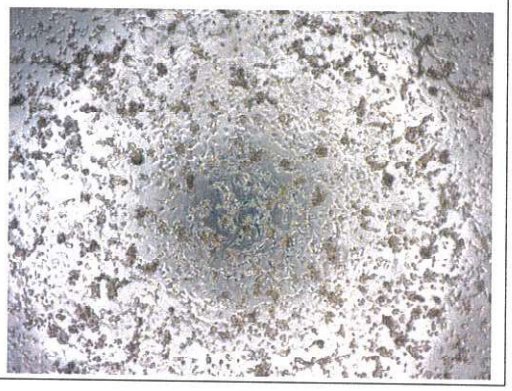
Goodness of fit: R^2= 0.8765
Best fit values:
Hill slope= 0.3288
[redacted]

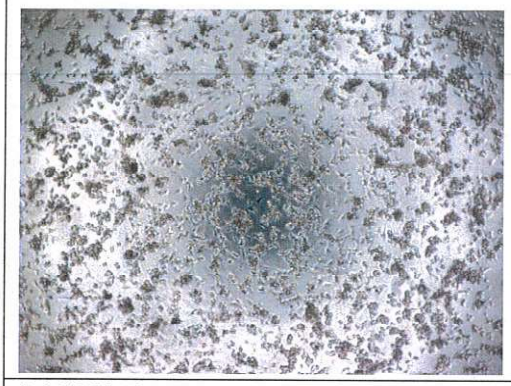
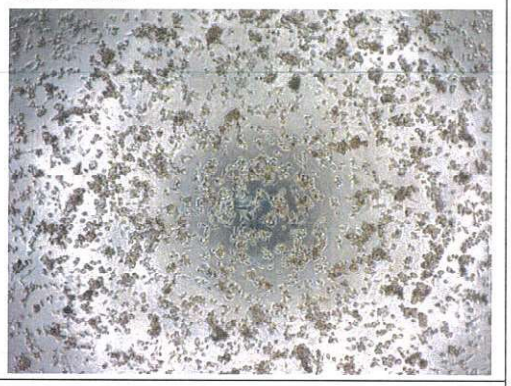
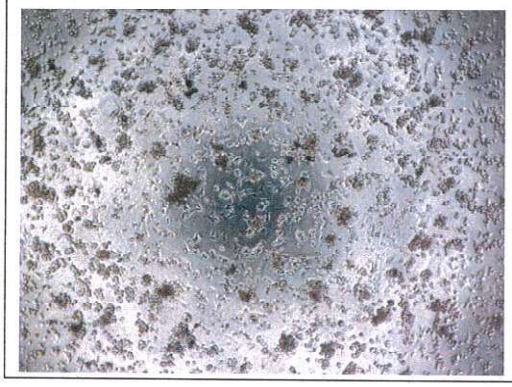
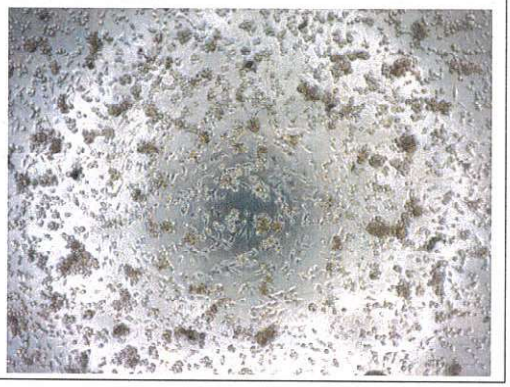
(三) [VELDONA®]細胞病變顯微鏡檢查(100X)

Cell control 	Virus control 
100000 U/ml 	20000 U/ml
4000 U/ml	800 U/ml 
160 U/ml 	32 U/ml

6.4 U/ml 	1.28 U/ml 
0.25 U/ml 	0.0512 U/ml 

(四) [Internal control] 細胞病變顯微鏡檢查(100X)

Cell control 	Virus control 
100000 U/ml 	20000 U/ml 
4000 U/ml 	800 U/ml 
160 U/ml 	32 U/ml 

6.4 U/ml 	1.28 U/ml 
0.25 U/ml 	0.0512 U/ml 

細胞毒性

(一) MTS/PMS OD 值及相對百分比

		VELDONA® (Mean ± SD, n=6)		Internal control (Mean ± SD, n=6)	
		OD	(%)	OD	(%)
Test sample concentration (U/ml)	0	1.397±0.019	100±1.39	1.377±0.016	100±1.19
	0.0512	1.448±0.015	103.64±1.10	1.445±0.032	104.99±2.33
	0.25	[redacted]			
	1.28	[redacted]			
	6.4	1.487±0.024	106.42±1.72	1.433±0.015	104.1±1.1
	32	[redacted]			
	160	[redacted]			
	800	1.255±0.031	89.87±2.19	1.160±0.03	84.26±2.19
	4000	[redacted]			
	20000	[redacted]			
	100000	1.213±0.038	86.80±2.69	1.131±0.017	82.15±1.27

三、數據判讀:

1、 樣品取連續 ≥6 個濃度之存活率數值，且在 0%~50%與 50%~100%區間各需至少包含一濃度數值。所得劑量反應曲線圖之 R^2>0.85。

2、 本次測試結果顯示 VELDONA® 之 EC50 為 1062 U/ml，Internal control 之 EC50 為 38.81 U/ml，兩者數值相差 27.7 倍。

細胞工程實驗室
實驗執行人員：鍾玉東　　實驗室負責人：高增祿
2022.11.15