

# **PBL Assay Science Assay Services—Sample Report**

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Dear Kerry,

## Assay Services – Sample Report

This report represents a sample of the typical assays performed and data generated by PBL's Assay Services. All services are tailored to customer's specific requirements including standards, references and number of replicates included. Assays are developed for research use or to a standard which supports subsequent GLP validation. All of our assay services are provided under confidentiality.

For further details on our Assay Services capabilities please visit our website at [www.pbl assaysci.com](http://www.pbl assaysci.com), contact us at telephone number 1-732-777-9123 or e:mail: [assayservices@pblbio.com](mailto:assayservices@pblbio.com).

### Summary:

**Interferon- $\alpha$  Cytopathic Effect Assay:** Test articles are run in duplicate in a viral challenge assay using EMC virus on A549 cells. Plates are stained with crystal violet, a visual CPE determination is performed and the dye is solubilized and absorbance read. These data are then analyzed in Graph Pad Prism using a sigmoidal fit (variable slope).

**Neutralizing Antibody Assay:** Samples are tested in duplicate in a neutralization assay. Briefly, antibody samples are titrated against a known interferon concentration. One neutralization unit is the amount of antibody required to neutralize one unit of interferon to a 50% endpoint. The units are determined with respect to a reference standard calibrated to the international reference standard. Human interferon challenge titration and standard titration are conducted on the same assay plate.

**Proliferation Assays:** GM-CSF is tested in duplicate in a proliferation assay using TF-1 cells. Cells proliferated for 2 days prior to signal development. IL-4 is tested in duplicate in a proliferation assay using TF-1 cells. Cells proliferated for 4 days prior to signal development. Cellular proliferation is quantified using Promega MTS (cat# G5430) and absorbance at 490 nm read. These data are then analyzed in GraphPad Prism using a sigmoidal fit (variable slope).

**Anti-Proliferation Assay:** Customer samples, PBL IFN $\alpha$ 2A and NIBSC 95/650 (WHO International Standard) are tested in duplicate in anti-proliferation assays using Ovar-3 cells. Anti-proliferation is quantified using Promega MTS (cat# G5430) and absorbance at 490 nm read. These data are then analyzed in Graph Pad Prism using a sigmoidal fit (variable slope). For the Ovar-3 proliferation assays, the bottom portion of the curve is constrained to zero (% maximum).

**Cytokine Release Assay:** NIBSC 95/650 is tested in duplicate in an IFN- $\gamma$  release assay using NK-92 cells. Briefly, NK-92 cells are treated with NIBSC 95/650 (IFN $\alpha$ ) for 24 hours. Supernatants are harvested and IFN- $\gamma$  release is quantified by ELISA. The amount of IFN- $\gamma$  released is plotted relative to IFN- $\alpha$  concentration and then analyzed in GraphPad Prism using a sigmoidal fit (variable slope).

**VeriPlex™ Human Multiplex ELISA:** Samples are tested in duplicate in the VeriPlex™ Human Interferon Multiplex ELISA kit (#51500-1). Data are analyzed using the Quansys Biosciences Q-View™ software. In brief, interferon and cytokine levels were determined by backfitting to standard curves generated using a 5-parameter curve fit. These values were exported into excel and listed in summary form. The summary table below represents overall interferon and cytokine signatures for each sample, as determined using the Q-View™ software. Values in bold represent calculated values above the limit of detection (LOD), as determined by the analysis software. Values that are above the LOD, but below the lower limit of quantitation (LLOQ) have been color-coded in violet.

Please do not hesitate to contact us with further questions.

Best Regards,

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### Interferon- $\alpha$ Cytopathic Effect Assay

Sample	Sample Dilution/ Volume	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)
1	1000000	8	954	9.5E+08	9.8E+08
	1000000	8	954		
2	4000	7.5	674	2.7E+06	3.4E+06
	4000	7.5	674		

### Neutralizing Antibody Assay

Sample	Sample Dilution/ Volume	Endpoint Well	Antibody Dilution at Endpoint	Visual Read Assay Std Titer (U/well)	Total Titer Visual Read (NU/ml)	Total Titer Graph Pad (NU/ml)
Antibody A	10	6.5	226	3.17	2.9E+04	2.6E+04
	10	6.5	226			
Antibody B	100	3.5	28.3	3.17	3.1E+04	2.9E+04
	100	3.0	20.0			

### TF-1 Proliferation Assay

Sample	EC <sub>50</sub> (pg/ml)
Commercial IL-4	148.5
Commercial GM-CSF	331.3

### Ovcar-3 Anti-Proliferation Assay

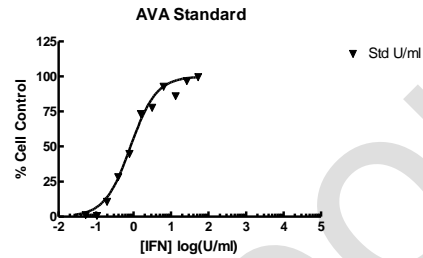
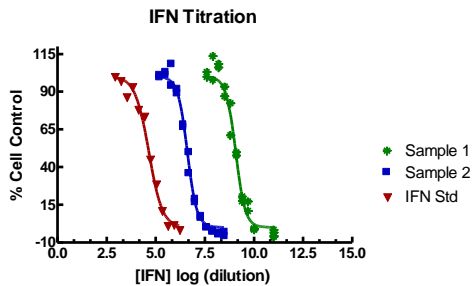
Sample	EC <sub>50</sub> (pg/ml)
PBL IFN-alpha 2A	1264
NIBSC 95/650 (IFNa2A)	1893

### NK-92 IFN-gamma Release Assay

Sample	EC <sub>50</sub> (pg/ml)
NIBSC 95/650 (IFNa2A)	484.3

# Interferon- $\alpha$ Cytopathic Effect Assay

	Sample 1	Sample 2	IFN Std	Std U/ml
EC50	1.18e+009	4.12e+006	44743	0.832



	Sample 1	Sample 2	STD	Std U/ml
<i>Sigmoidal dose-response (variable slope)</i>				
<i>Best-fit values</i>				
BOTTOM	0	0	0	0
TOP	100	100	100	100
LOGEC50	9.07	6.61	4.65	-0.0798
HILLSLOPE	-1.78	-1.79E+00	-1.26	1.27
<b>EC50</b>	<b>1.18E+09</b>	<b>4.12E+06</b>	<b>4.47E+04</b>	<b>0.832</b>

#### 95% Confidence Intervals

LOGEC50	9.01 to 9.14	6.57 to 6.66	4.57 to 4.73	-0.162 to 0.00263
HILLSLOPE	-2.19 to -1.38	-2.06 to -1.51	-1.52 to -1.00	0.998 to 1.53
EC50	1.02e+009 to 1.37e+009	3.74e+006 to 4.54e+006	37182 to 53841	0.688 to 1.01

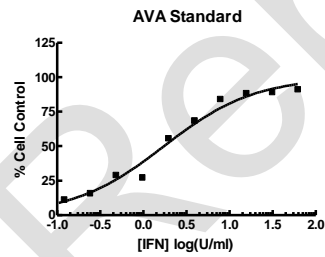
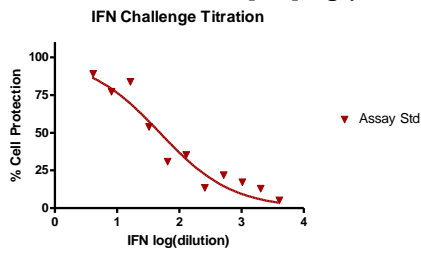
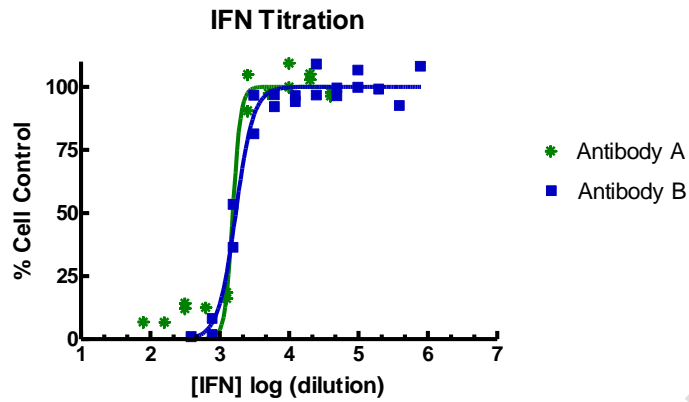
#### Goodness of Fit

Degrees of Freedom	22	22	10	10
R <sup>2</sup>	0.98	9.91E-01	0.989	0.988
Absolute Sum of Squ.	957	415	210	222
Sy,x	6.6	4.34	4.58	4.71

#### Calculations for U/ml and SA

	Sample 1	Sample 2	STD
U/ml	9.82E+08	3.43E+06	37226.176

# Neutralizing Antibody Assay



	10416	10416	Assay Std	Std	Std U/ml
Sigmoidal dose-response (variable slope)					
Best-fit values					
BOTTOM					
TOP	100	100	100	100	100
LOGEC50	3.19	3.23	1.68	3.16	0.24
HILLSLOPE	7.57E+00	3.42E+00	-0.729	-0.83	0.826
EC50	1.55E+03	1.71E+03	4.81E+01	1435	1.74
95% Confidence Intervals					
LOGEC50	3.10 to 3.28	3.17 to 3.29	1.49 to 1.88	3.03 to 3.28	0.117 to 0.362
HILLSLOPE	0.359 to 14.8	1.87 to 4.96	-0.954 to -0.505	-1.01 to -0.653	0.651 to 1.00
EC50	1256 to 1902	1491 to 1952	30.7 to 75.3	1082 to 1904	1.31 to 2.30

Calculations for NU/ml and SA

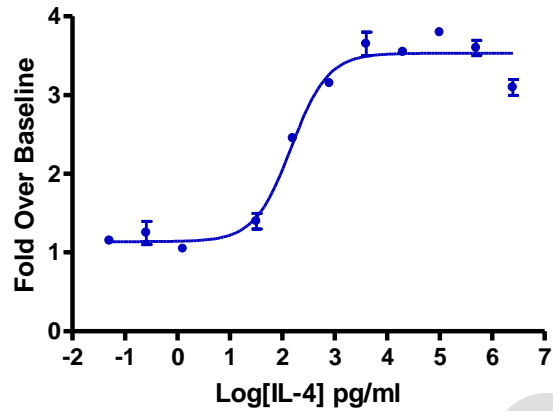
	Assay Std	Std
U/well	4.18	2.50E+03

	10416	10416
NU/ml	2.59E+04	2.86E+04

Sample	10416	10416
EC50 ng/ml	2270.38	2057.44
NU/mg	7.37E+03	8.14E+03

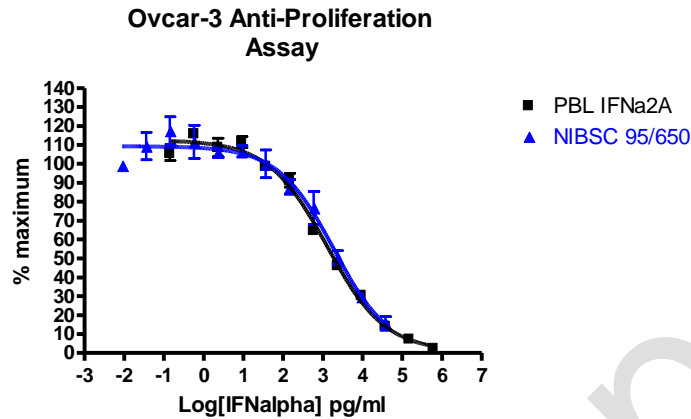
# TF-1 Cellular Proliferation Assay

## TF-1 Proliferation Assay: IL-4



Commercial IL-4	
<i>Sigmoidal dose-response (variable slope)</i>	
<b>Best-fit values</b>	
BOTTOM	1.139
TOP	3.532
LOGEC50	2.172
HILLSLOPE	1.243
EC50	148.5
<b>Std. Error</b>	
BOTTOM	0.0879
TOP	0.07055
LOGEC50	0.08869
HILLSLOPE	0.291
<b>95% Confidence Intervals</b>	
BOTTOM	0.9547 to 1.324
TOP	3.384 to 3.680
LOGEC50	1.985 to 2.358
HILLSLOPE	0.6312 to 1.854
EC50	96.71 to 228.1
<b>Goodness of Fit</b>	
Degrees of Freedom	18
R <sup>2</sup>	0.9675
Absolute Sum of Squares	0.8353
Sy.x	0.2154

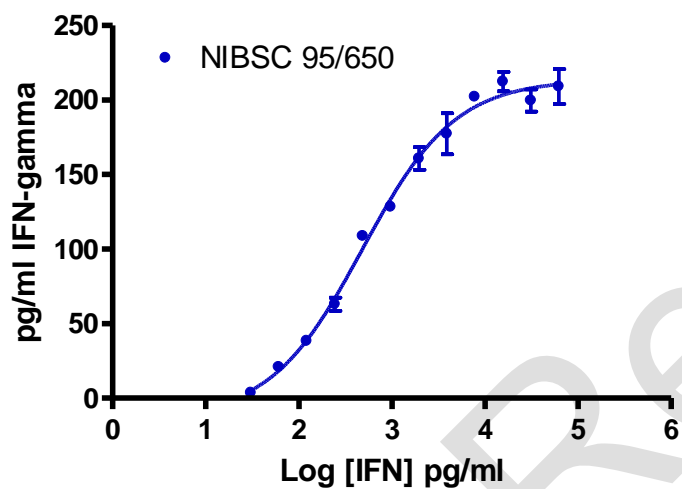
# Ovcar-3 Anti-Proliferation Assay



	PBL IFNa2A	NIBSC 95/650
<i>Sigmoidal dose-response (variable slope)</i>		
<b>Best-fit values</b>		
BOTTOM	0.743	0.5475
TOP	112.6	109.3
LOGEC50	3.102	3.277
HILLSLOPE	-0.5875	-0.617
EC50	1264	1893
<b>Std. Error</b>		
BOTTOM	3.468	15.49
TOP	2.153	2.599
LOGEC50	0.08124	0.2615
HILLSLOPE	0.06105	0.1467
<b>95% Confidence Intervals</b>		
BOTTOM	-6.490 to 7.976	-31.76 to 32.86
TOP	108.1 to 117.1	103.9 to 114.7
LOGEC50	2.932 to 3.271	2.732 to 3.823
HILLSLOPE	-0.7149 to -0.4602	-0.9230 to -0.3111
EC50	855.7 to 1867	539.1 to 6650
<b>Goodness of Fit</b>		
Degrees of Freedom	20	20
R <sup>2</sup>	0.9899	0.9559
Absolute Sum of Squares	437.8	1163
Sy.x	4.679	7.625



## NK-92 IFN $\gamma$ release assay



	NIBSC 95/650
<i>Sigmoidal dose-response (variable slope)</i>	
<b>Best-fit values</b>	
BOTTOM	-13.65
TOP	213.8
LOGEC50	2.685
HILLSLOPE	0.8723
EC50	484.3
<b>Std. Error</b>	
BOTTOM	10.46
TOP	4.664
LOGEC50	0.05792
HILLSLOPE	0.09518
<b>95% Confidence Intervals</b>	
BOTTOM	-35.47 to 8.174
TOP	204.1 to 223.5
LOGEC50	2.564 to 2.806
HILLSLOPE	0.6738 to 1.071
EC50	366.7 to 639.6
<b>Goodness of Fit</b>	
Degrees of Freedom	20
R <sup>2</sup>	0.9911
Absolute Sum of Squares	1204
Sy.x	7.758

## Veriplex Multiplex Human IFN ELISA

Sample	IFN alpha	IFN beta	IFN gamma	IFN lambda	IFN omega	IL-1alpha	IL-6	IP-10	TNFalpha
Control - 1	4.89	35.91	10.07	18.89	8.29	3.28	32.22	3.26	5.47
Virus induced - 1	1322.21	454.99	55.04	109.96	159.22	3.74	319.00	51.42	261.25
TLR agonist A - 1	64.79	37.53	10.51	10.12	14.08	0.53	30.63	23.15	6.82
TLR agonist B - 1	4.94	39.70	10.05	42.14	6.97	1.60	34.51	7.67	5.24
TLR agonist C - 1	5.70	35.91	10.23	18.07	6.91	1.23	37.82	39.46	7.39
TLR agonist D - 1	10.33	36.23	10.33	34.90	6.94	15.74	4200.85	11.05	955.56
TLR agonist E - 1	4.47	36.85	10.14	6.28	7.12	1.52	38.40	1.88	13.39
TLR mixture - 1	12.97	44.28	12.60	51.37	8.83	60.19	6434.56	12.94	2340.95
Control - 2	4.39	34.33	9.50	26.97	7.02	0.92	32.10	4.00	4.77
Virus induced - 2	1888.46	1175.90	62.12	308.40	298.03	9.54	4644.68	178.04	1566.79
TLR agonist A - 2	274.42	38.62	10.38	12.61	57.49	0.00	32.08	350.58	10.89
TLR agonist B - 2	4.72	36.36	10.98	53.92	6.90	2.24	43.23	102.60	6.35
TLR agonist C - 1	19.02	39.92	13.22	9.65	6.74	12.40	556.04	458.75	34.51
TLR agonist D - 2	10.55	34.05	13.54	29.07	6.82	79.13	9041.26	18.24	>3000
TLR agonist E - 2	4.75	36.77	11.36	26.48	7.45	2.67	386.03	5.39	132.50
TLR mixture - 2	30.05	50.72	49.56	51.92	7.81	351.87	21663.37	44.05	>3000

### Std Curve

Upper Limit	3500	19000	5400	8000	4050	1100	20000	1500	3000
Lower Limit	4.8	26.1	7.4	11	5.6	1.5	27.4	2.1	4.1

### Assay Information

Limit of Detection	0.7	32.9	12.4	37.5	0.1	1.5	22.4	3.6	1.0
Lower Limit of Quantification	13.7	84.3	20.5	98.6	15.4	12.1	86.9	7.3	11.9
Upper Limit of Quantification	3423.7	18980.4	5390.7	8000.0	4044.9	1100.0	17448.6	1496.6	2984.4

- Values in bold represent results calculated above the limit of detection with CV values within acceptable limits (<51%). Shading indicates results which are above the limit of detection, but below the limit of quantification.