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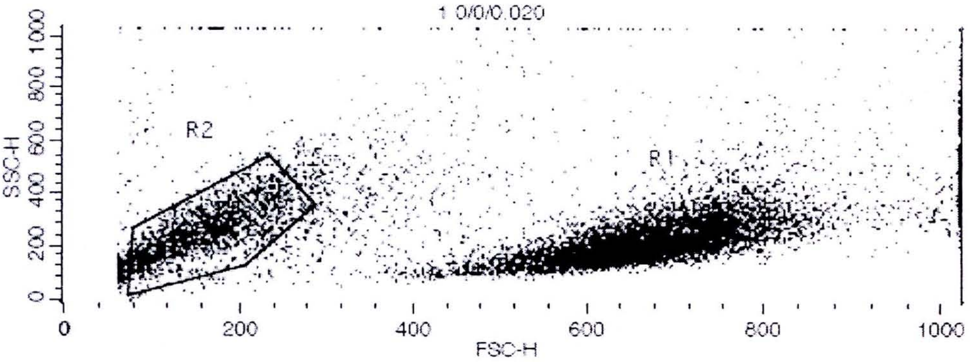
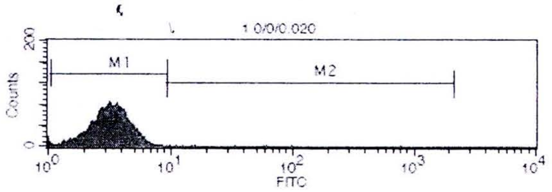
# APPENDIXES

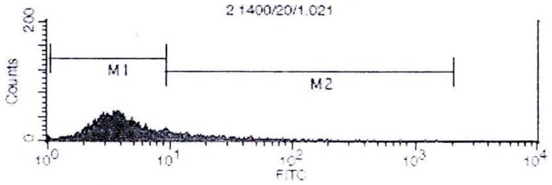
# APPENDIX A The Expression of EGFP After Electrotransfer in Jurkat Cells.





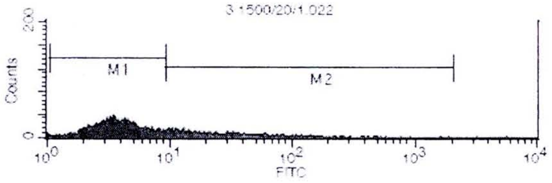
File: 1 0/0/0.020 Gate: G1
Gated Events: 6547 Total Events: 20000
X Parameter: FITC (Log)

| Marker | Events | % Gated | % Total | Mean | Geo Mean |
|---|---|---|---|---|---|
| All | 6547 | 100.00 | 32.73 | 3.54 | 3.20 |
| M1 | 6516 | 99.53 | 32.58 | 3.37 | 3.20 |
| M2 | 14 | 0.21 | 0.07 | 83.69 | 28.46 |



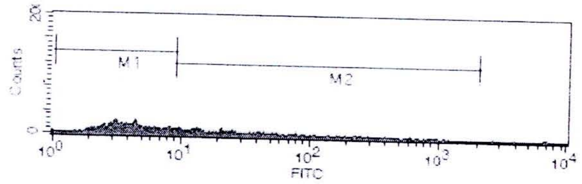
File: 2 1400/20/1.021 Gate: G1
Gated Events: 5284 Total Events: 20000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 5284 | 100.00 | 26.42 | 8.38 |
| M1 | 4367 | 82.65 | 21.83 | 3.95 |
| M2 | 953 | 18.04 | 4.76 | 28.77 |



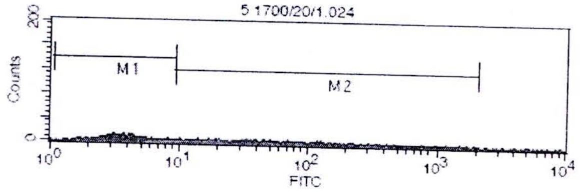
File: 3 1500/20/1.022 Gate: G1
Gated Events: 4408 Total Events: 20000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 4408 | 100.00 | 22.04 | 16.74 |
| M1 | 3121 | 70.80 | 15.60 | 4.03 |
| M2 | 1301 | 29.51 | 6.50 | 40.81 |



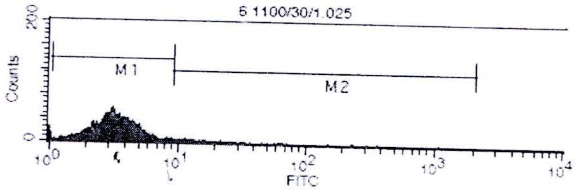
File: 4 1600/20/1.023 Gate: G1
Gated Events: 2876 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 2876 | 100.00 | 19.17 | 44.85 |
| M1 | 1693 | 58.87 | 11.29 | 4.22 |
| M2 | 1189 | 41.34 | 7.93 | 72.80 |



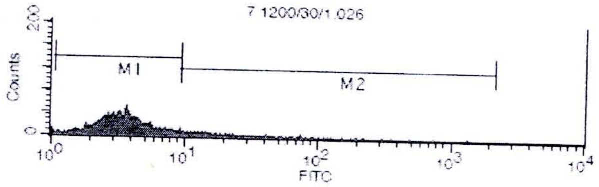
File: 5 1700/20/1.024 Gate: G1
Gated Events: 1963 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 1963 | 100.00 | 13.09 | 174.75 |
| M1 | 839 | 42.74 | 5.59 | 4.18 |
| M2 | 1103 | 56.19 | 7.35 | 187.00 |



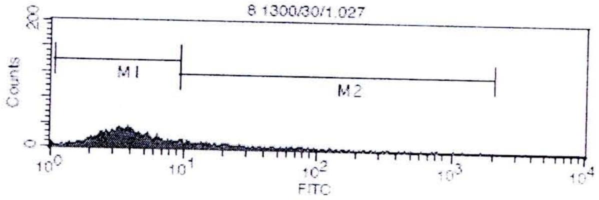
File: 6 1100/30/1.025 Gate: G1
Gated Events: 4027 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 4027 | 100.00 | 26.85 | 4.75 |
| M1 | 3796 | 94.26 | 25.31 | 3.53 |
| M2 | 217 | 5.39 | 1.45 | 26.71 |



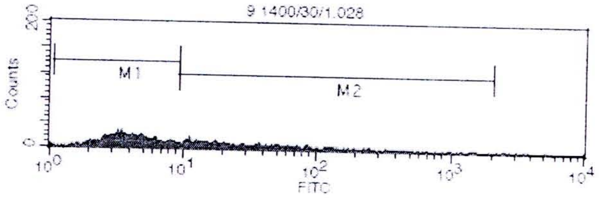
File: 7 1200/30/1.026 Gate: G1
Gated Events: 4037 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 4037 | 100.00 | 26.91 | 6.96 |
| M1 | 3524 | 87.29 | 23.49 | 3.70 |
| M2 | 491 | 12.16 | 3.27 | 30.49 |



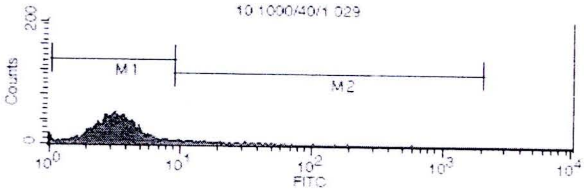
File: 8 1300/30/1.027 Gate: G1
Gated Events: 3560 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3560 | 100.00 | 23.73 | 12.22 |
| M1 | 2754 | 77.36 | 18.36 | 3.94 |
| M2 | 831 | 23.34 | 5.54 | 39.64 |



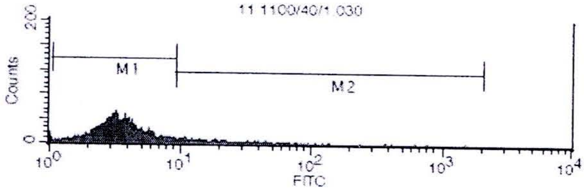
File: 9 1400/30/1.028 Gate: G1
Gated Events: 3259 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3259 | 100.00 | 21.73 | 49.01 |
| M1 | 1984 | 60.88 | 13.23 | 4.21 |
| M2 | 1271 | 39.00 | 8.47 | 72.48 |



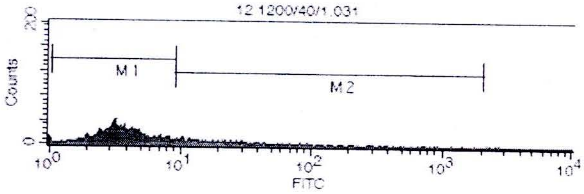
File: 10 1000/40/1.029 Gate: G1
Gated Events: 3890 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3890 | 100.00 | 25.93 | 4.36 |
| M1 | 3691 | 94.88 | 24.61 | 3.43 |
| M2 | 187 | 4.81 | 1.25 | 23.20 |



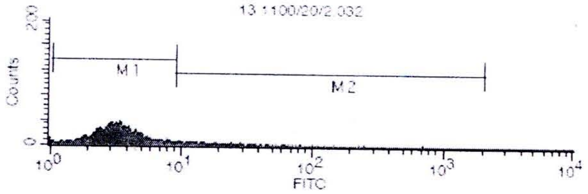
File: 11 1100/40/1.030 Gate: G1
Gated Events: 3894 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3894 | 100.00 | 25.96 | 6.71 |
| M1 | 3458 | 88.80 | 23.05 | 3.70 |
| M2 | 431 | 11.07 | 2.87 | 31.13 |



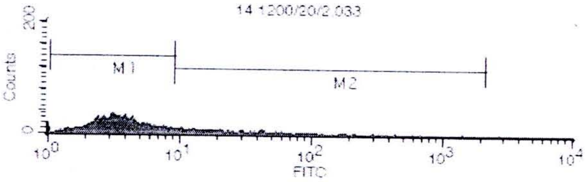
File: 12 1200/40/1.031 Gate: G1
Gated Events: 3309 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3309 | 100.00 | 22.06 | 17.82 |
| M1 | 2550 | 77.06 | 17.00 | 3.87 |
| M2 | 757 | 22.88 | 5.05 | 49.67 |



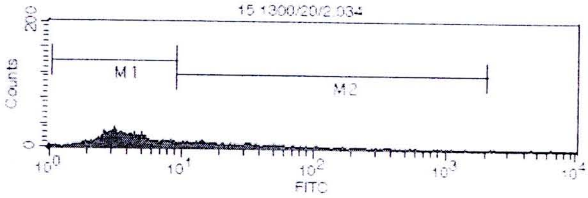
File: 13 1100/20/2.032 Gate: G1
Gated Events: 2873 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 2873 | 100.00 | 19.15 | 5.20 |
| M1 | 2657 | 92.48 | 17.71 | 3.56 |
| M2 | 214 | 7.45 | 1.43 | 25.84 |



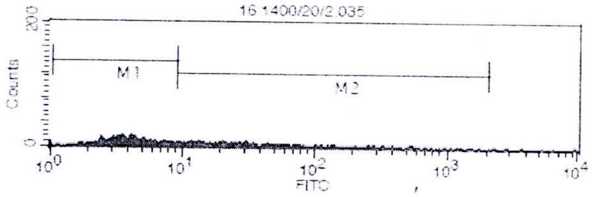
File: 14 1200/20/2.033 Gate: G1
Gated Events: 3514 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3514 | 100.00 | 23.43 | 10.06 |
| M1 | 2864 | 81.50 | 19.09 | 3.85 |
| M2 | 628 | 17.87 | 4.19 | 34.51 |



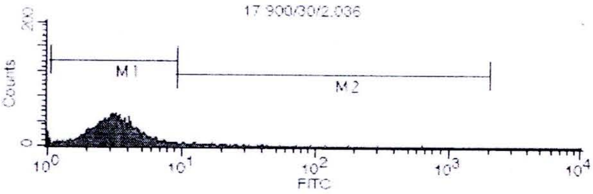
File: 15 1300/20/2.034 Gate: G1
Gated Events: 2976 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 2976 | 100.00 | 19.84 | 23.82 |
| M1 | 2021 | 67.91 | 13.47 | 4.02 |
| M2 | 961 | 32.29 | 6.41 | 50.11 |



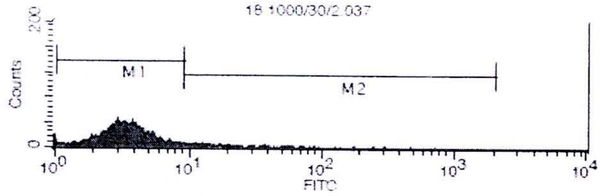
File: 16 1400/20/2.035 Gate: G1
Gated Events: 2377 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 2377 | 100.00 | 15.85 | 79.79 |
| M1 | 1278 | 53.77 | 8.52 | 4.26 |
| M2 | 1092 | 45.94 | 7.28 | 109.00 |



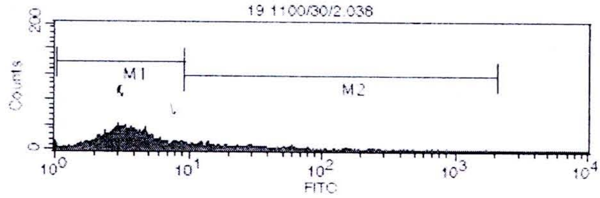
File: 17 900/30/2.036 Gate: G1
Gated Events: 4018 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 4018 | 100.00 | 26.79 | 3.97 |
| M1 | 3894 | 96.91 | 25.96 | 3.36 |
| M2 | 110 | 2.74 | 0.73 | 26.49 |



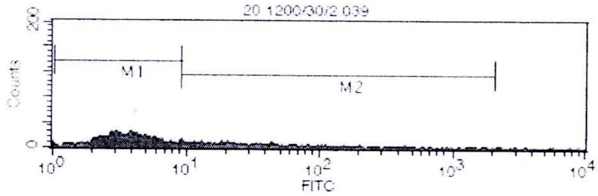
File: 18 1000/30/2.037 Gate: G1
Gated Events: 3695 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3695 | 100.00 | 24.63 | 8.29 |
| M1 | 3404 | 92.12 | 22.69 | 3.61 |
| M2 | 283 | 7.66 | 1.89 | 30.05 |



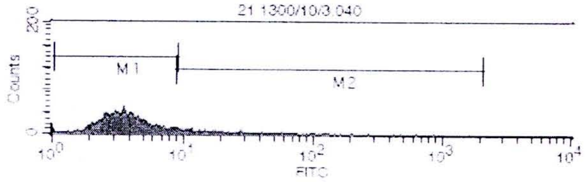
File: 19 1100/30/2.038 Gate: G1
Gated Events: 3973 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3973 | 100.00 | 26.49 | 12.65 |
| M1 | 3218 | 81.00 | 21.45 | 3.82 |
| M2 | 763 | 19.20 | 5.09 | 37.04 |



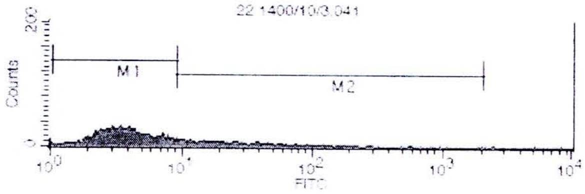
File: 20 1200/30/2.039 Gate: G1
Gated Events: 3059 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3059 | 100.00 | 20.39 | 50.56 |
| M1 | 2085 | 68.16 | 13.90 | 4.02 |
| M2 | 972 | 31.78 | 6.48 | 96.09 |



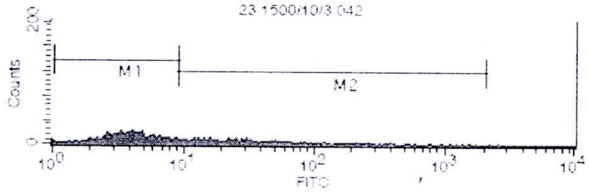
File: 21 1300/10/3.040 Gate: G1
Gated Events: 4048 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 4048 | 100.00 | 26.99 | 7.17 |
| M1 | 3475 | 85.84 | 23.17 | 3.83 |
| M2 | 581 | 14.35 | 3.87 | 27.43 |



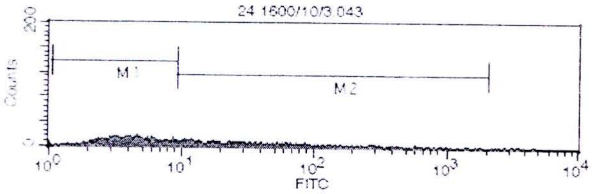
File: 22 1400/10/3.041 Gate: G1
Gated Events: 3873 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 3873 | 100.00 | 25.82 | 19.92 |
| M1 | 2914 | 75.24 | 19.43 | 3.98 |
| M2 | 970 | 25.05 | 6.47 | 44.92 |



File: 23 1500/10/3.042 Gate: G1
Gated Events: 2825 Total Events: 15000

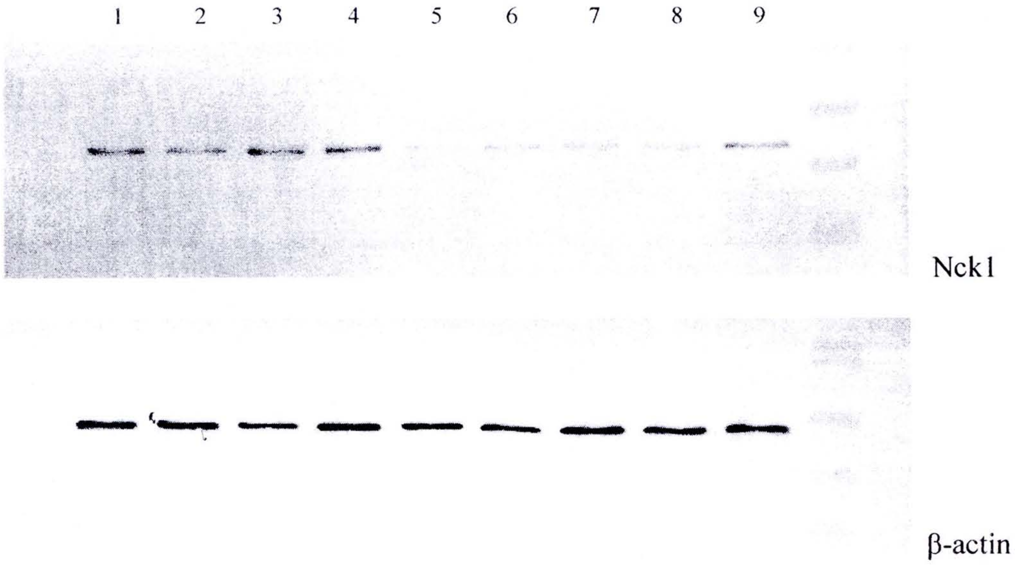
| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 2825 | 100.00 | 18.83 | 26.50 |
| M1 | 1769 | 62.62 | 11.79 | 4.20 |
| M2 | 1059 | 37.49 | 7.06 | 59.49 |

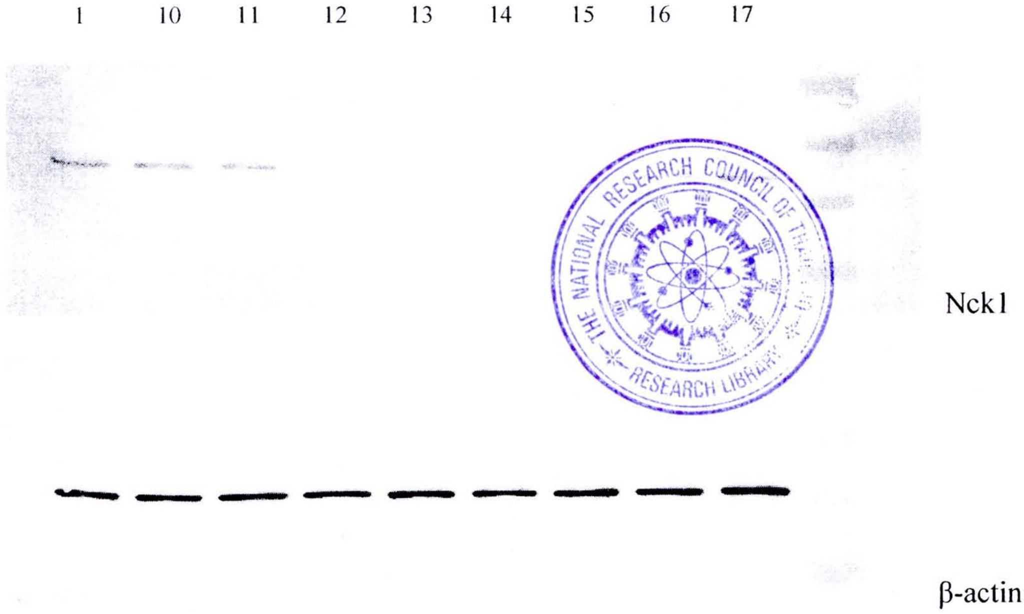


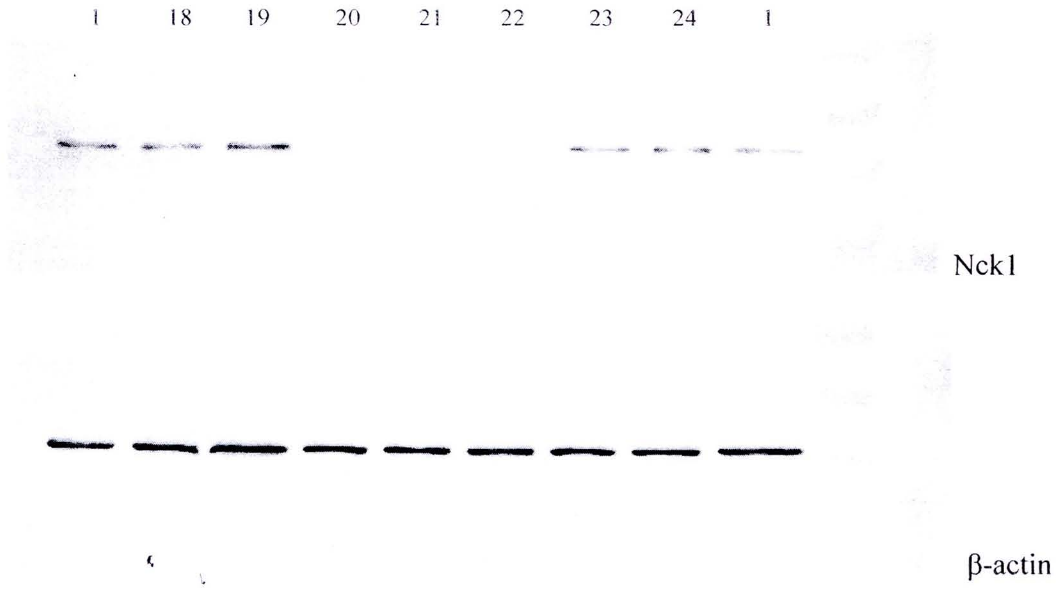
File: 24 1600/10/3.043 Gate: G1
Gated Events: 2926 Total Events: 15000

| Marker | Events | % Gated | % Total | Mean |
|---|---|---|---|---|
| All | 2926 | 100.00 | 19.51 | 61.44 |
| M1 | 1489 | 50.89 | 9.93 | 4.33 |
| M2 | 1436 | 49.08 | 9.57 | 87.62 |

## APPENDIX B Western Blot Analysis of Nck1 Protein Expression.







Lane 1: blank control in which the cells were not treated.
Lane 2: the cells were transfected with pEGFP at 1700 V, 20 msec, 1.
Lane 3: the cells were treated with 100 pmol Nck1 siRNA.
Lane 4: the cells were treated with 50 pmol Nck1 siRNA.
Lane 5: the cells were transfected with 100 pmol Nck1 siRNA at 1700 V, 20 msec, 1.
Lane 6: the cells were transfected with 50 pmol Nck1 siRNA at 1700 V, 20 msec, 1.
Lane 7: the cells were transfected with 25 pmol Nck1 siRNA at 1700 V, 20 msec, 1.
Lane 8: the cells were transfected with 12.5 pmol Nck1 siRNA at 1700 V, 20 msec, 1.
Lane 9: the cells were transfected with 100 pmol negative control siRNA at 1700 V, 20 msec, 1.
Lane 10: the cells were transfected with 50 pmol negative control siRNA at 1700 V, 20 msec, 1.
Lane 11: the cells were transfected with pEGFP at 1600 V, 10 msec, 3.
Lane 12: the cells were transfected with 100 pmol Nck1 siRNA at 1600 V, 10 msec, 3.
Lane 13: the cells were transfected with 50 pmol Nck1 siRNA at 1600 V, 10 msec, 3.
Lane 14: the cells were transfected with 25 pmol Nck1 siRNA at 1600 V, 10 msec, 3.
Lane 15: the cells were transfected with 12.5 pmol Nck1 siRNA at 1600 V, 10 msec, 3.
Lane 16: the cells were transfected with 100 pmol negative control siRNA at 1600 V, 10 msec, 3.
Lane 17: the cells were transfected with 50 pmol negative control siRNA at 1600 V, 10 msec, 3.
Lane 18: the cells were transfected with pEGFP at 1400V, 20 msec, 2.
Lane 19: the cells were transfected with 100 pmol Nck1 siRNA at 1400V, 20 msec, 2.
Lane 20: the cells were transfected with 50 pmol Nck1 siRNA at 1400V, 20 msec, 2.
Lane 21: the cells were transfected with 25 pmol Nck1 siRNA at 1400V, 20 msec, 2.
Lane 22: the cells were transfected with 12.5 pmol Nck1 siRNA at 1400V, 20 msec, 2.
Lane 23: the cells were transfected with 100 pmol negative control siRNA at 1400V,

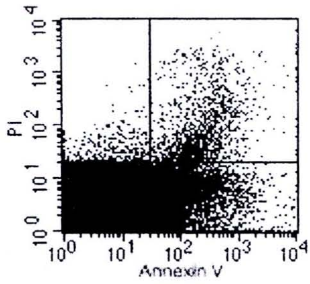
20 msec, 2.

Lane 24: the cells were transfected with 50 pmol negative control siRNA 1400V, 20 msec, 2.

## APPENDIX C The Influence of Nck1 siRNA on Jurkat T Cell Apoptosis.

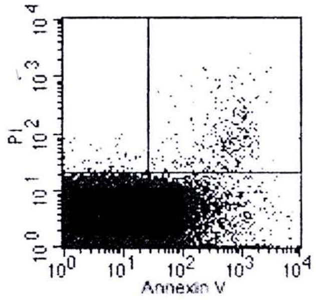
### Experiment 1.

(A)



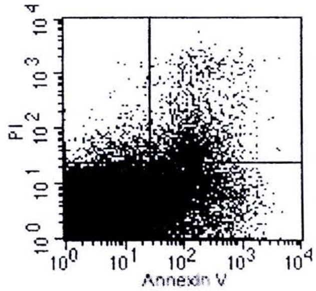
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 345 | 0.34 | 0.34 |
| UR | 1365 | 1.36 | 1.36 |
| LL | 87985 | 87.98 | 87.98 |
| LR | 10305 | 10.30 | 10.30 |

(B)



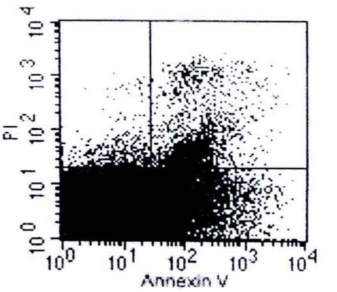
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 68 | 0.07 | 0.07 |
| UR | 312 | 0.31 | 0.31 |
| LL | 90243 | 90.24 | 90.24 |
| LR | 9377 | 9.38 | 9.38 |

(C)



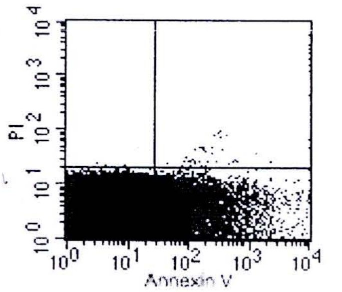
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 532 | 0.53 | 0.53 |
| UR | 1781 | 1.78 | 1.78 |
| LL | 89183 | 89.18 | 89.18 |
| LR | 8504 | 8.50 | 8.50 |

(D)



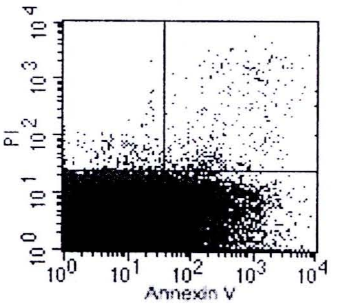
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 475 | 0.47 | 0.47 |
| UR | 2079 | 2.08 | 2.08 |
| LL | 84655 | 84.66 | 84.66 |
| LR | 12791 | 12.79 | 12.79 |

(E)



| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 5 | 0.01 | 0.01 |
| UR | 55 | 0.06 | 0.06 |
| LL | 86949 | 86.95 | 86.95 |
| LR | 12991 | 12.99 | 12.99 |

(F)

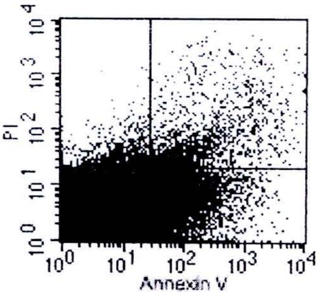


| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 230 | 0.23 | 0.23 |
| UR | 380 | 0.38 | 0.38 |
| LL | 85101 | 85.10 | 85.10 |
| LR | 14289 | 14.29 | 14.29 |

E6-1 Jurkat T cells were transfected with Nck1-specific siRNA (A), left untransfected (B), or transfected with non-specific siRNA negative control (C). TCR-stimulated Jurkat cells were transfected with Nck1-specific siRNA (D), left untransfected (E), or transfected with non-specific siRNA negative control (F).

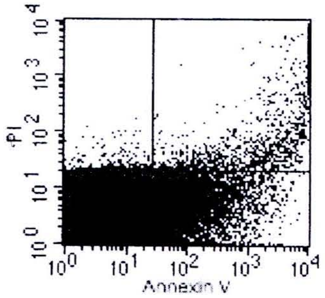
**Experiment 2.**

(A)



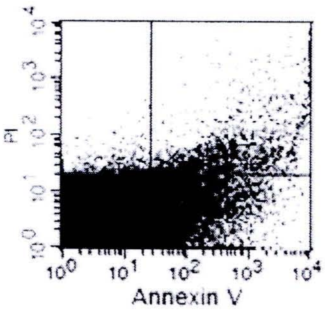
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 1461 | 0.73 | 0.73 |
| UR | 2348 | 1.17 | 1.17 |
| LL | 176313 | 88.16 | 88.16 |
| LR | 19878 | 9.94 | 9.94 |

(B)



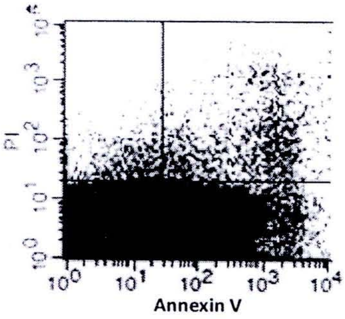
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 86 | 0.03 | 0.03 |
| UR | 865 | 0.35 | 0.35 |
| LL | 226967 | 90.79 | 90.79 |
| LR | 22082 | 8.83 | 8.83 |

(C)



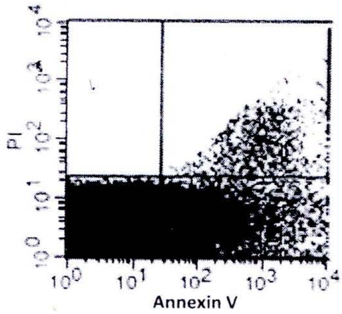
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 434 | 0.22 | 0.22 |
| UR | 1891 | 0.95 | 0.95 |
| LL | 178708 | 89.35 | 89.35 |
| LR | 18967 | 9.48 | 9.48 |

(D)



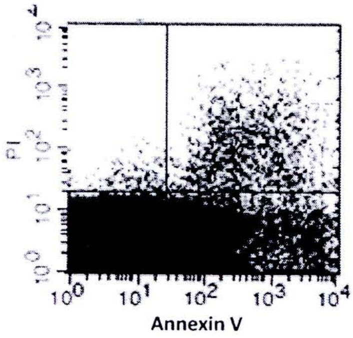
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 692 | 0.28 | 0.28 |
| UR | 4964 | 1.99 | 1.99 |
| LL | 216415 | 86.57 | 86.57 |
| LR | 27909 | 11.16 | 11.16 |

(E)



| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 1 | 0.00 | 0.00 |
| UR | 1190 | 0.48 | 0.48 |
| LL | 221810 | 88.72 | 88.72 |
| LR | 26999 | 10.80 | 10.80 |

(F)

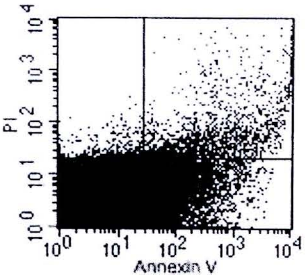


| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 154 | 0.06 | 0.06 |
| UR | 1598 | 0.64 | 0.64 |
| LL | 219700 | 87.88 | 87.88 |
| LR | 28548 | 11.42 | 11.42 |

E6-1 Jurkat T cells were transfected with Nck1-specific siRNA (A), left untransfected (B), or transfected with non-specific siRNA negative control (C). TCR-stimulated Jurkat cells were transfected with Nck1-specific siRNA (D), left untransfected (E), or transfected with non-specific siRNA negative control (F).

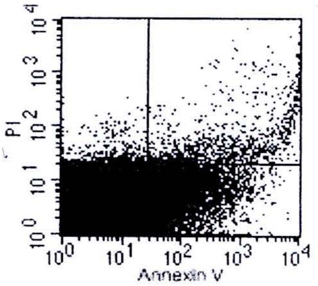
**Experiment 3.**

(A)



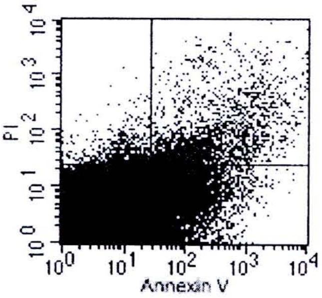
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 542 | 0.27 | 0.27 |
| UR | 2059 | 1.03 | 1.03 |
| LL | 174197 | 87.10 | 87.10 |
| LR | 23202 | 11.60 | 11.60 |

(B)



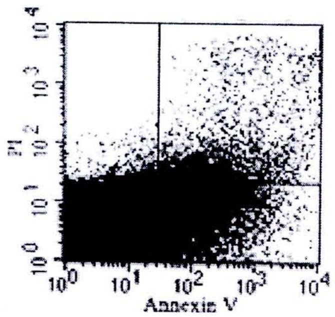
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 227 | 0.09 | 0.09 |
| UR | 558 | 0.22 | 0.22 |
| LL | 223364 | 89.35 | 89.35 |
| LR | 25851 | 10.34 | 10.34 |

(C)



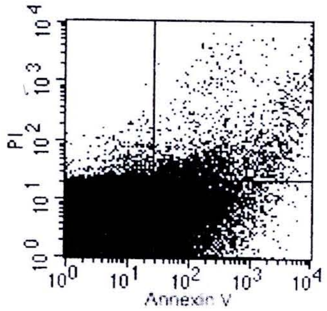
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 782 | 0.39 | 0.39 |
| UR | 2646 | 1.32 | 1.32 |
| LL | 176326 | 88.16 | 88.16 |
| LR | 20246 | 10.12 | 10.12 |

(D)



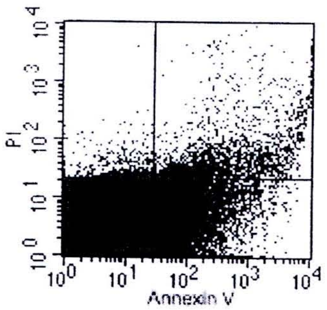
| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 443 | 0.18 | 0.18 |
| UR | 1727 | 0.69 | 0.69 |
| LL | 208971 | 83.59 | 83.59 |
| LR | 38859 | 15.54 | 15.54 |

(E)



| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 876 | 0.44 | 0.44 |
| UR | 2333 | 1.17 | 1.17 |
| LL | 169329 | 84.66 | 84.66 |
| LR | 27462 | 13.73 | 13.73 |

(F)



| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 709 | 0.35 | 0.35 |
| UR | 2528 | 1.26 | 1.26 |
| LL | 168726 | 84.36 | 84.36 |
| LR | 28037 | 14.02 | 14.02 |

E6-1 Jurkat T cells were transfected with Nck1-specific siRNA (A), left untransfected (B), or transfected with non-specific siRNA negative control (C). TCR-stimulated Jurkat cells were transfected with Nck1-specific siRNA (D), left untransfected (E), or transfected with non-specific siRNA negative control (F).

## Data Analysis

The data were expressed as the mean with a standard deviation (SD). Statistical data were analyzed with SPSS ANOVA followed by Dunnett's comparison test. The significance criterion for the correlation measurements was set at 0.05

### 1. Unstimulated cells

Univariate Analysis of Variance

Between-Subjects Factors

| | | Value Label | N |
|---|---|---|---|
| Cell condition | 1 | Cell | 3 |
| | 2 | Cell+Neg. | 3 |
| | 3 | Cell+siRNA | 3 |

Descriptive Statistics

Dependent Variable:result

| Cell condition | Mean | Std. Deviation | N |
|---|---|---|---|
| Cell | 9.81000 | .697782 | 3 |
| Cell+Neg. | 1.07167E1 | .630899 | 3 |
| Cell+siRNA | 1.18000E1 | .769610 | 3 |
| Total | 1.07756E1 | 1.055357 | 9 |

Levene's Test of Equality of Error Variances[a]

Dependent Variable:result

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .042 | 2 | 6 | .959 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + cell

Tests of Between-Subjects Effects

Dependent Variable:result

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 5.956[a] | 2 | 2.978 | 6.048 | .036 |
| Intercept | 1045.013 | 1 | 1045.013 | 2.122E3 | .000 |
| cell | 5.956 | 2 | 2.978 | 6.048 | .036 |
| Error | 2.954 | 6 | .492 | | |
| Total | 1053.924 | 9 | | | |
| Corrected Total | 8.910 | 8 | | | |

a. R Squared = .668 (Adjusted R Squared = .558)

Post Hoc Tests

Multiple Comparisons

| | | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| Tukey HSD | Cell | Cell+Neg. | -.90667 | .572952 | .323 | -2.66464 | .85131 |
| | | Cell+siRNA | -1.99000* | .572952 | .051 | -3.74797 | -.23203 |
| | Cell+Neg. | Cell | .90667 | .572952 | .323 | -.85131 | 2.66464 |
| | | Cell+siRNA | -1.08333 | .572952 | .221 | -2.84131 | .67464 |
| | Cell+siRNA | Cell | 1.99000* | .572952 | .051 | .23203 | 3.74797 |
| | | Cell+Neg. | 1.08333 | .572952 | .221 | -.67464 | 2.84131 |
| Scheffe | Cell | Cell+Neg. | -.90667 | .572952 | .351 | -2.74427 | .93094 |
| | | Cell+siRNA | -1.99000* | .572952 | .057 | -3.82761 | -.15239 |
| | Cell+Neg. | Cell | .90667 | .572952 | .351 | -.93094 | 2.74427 |
| | | Cell+siRNA | -1.08333 | .572952 | .246 | -2.92094 | .75427 |
| | Cell+siRNA | Cell | 1.99000* | .572952 | .057 | .15239 | 3.82761 |
| | | Cell+Neg. | 1.08333 | .572952 | .246 | -.75427 | 2.92094 |
| Dunnett t (2-sided)[a] | Cell | Cell+siRNA | -1.99000* | .572952 | .053 | -3.63022 | -.34978 |
| | Cell+Neg. | Cell+siRNA | -1.08333 | .572952 | .180 | -2.72355 | .55689 |

The error term is Mean Square(Error) = .492.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

result

| | Cell condition | N | Subset | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD[a] | Cell | 3 | 9.81000 | |
| | Cell+Neg. | 3 | 1.07167E1 | 1.07167E1 |
| | Cell+siRNA | 3 | | 1.18000E1 |
| | Sig. | | .323 | .221 |
| Scheffe[a] | Cell | 3 | 9.81000 | |
| | Cell+Neg. | 3 | 1.07167E1 | 1.07167E1 |
| | Cell+siRNA | 3 | | 1.18000E1 |
| | Sig. | | .351 | .246 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .492.

a. Uses Harmonic Mean Sample Size = 3.000.

Estimated Marginal Means

Cell condition

Dependent Variable:result

| Cell condition | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Cell | 9.810 | .405 | 8.819 | 10.801 |
| Cell+Neg. | 10.717 | .405 | 9.725 | 11.708 |
| Cell+siRNA | 11.800 | .405 | 10.809 | 12.791 |

## 2. Anti-TCR antibody stimulated cells

### Univariate Analysis of Variance

Between-Subjects Factors

| | | Value Label | N |
|---|---|---|---|
| Cell condition | 1 | Cell | 3 |
| | 2 | Cell+Neg. | 3 |
| | 3 | Cell+siRNA | 3 |

Descriptive Statistics

Dependent Variable:result

| Cell condition | Mean | Std. Deviation | N |
|---|---|---|---|
| Cell | 1.30767E1 | 1.810147 | 3 |
| Cell+Neg. | 1.40033E1 | 1.710390 | 3 |
| Cell+siRNA | 1.47500E1 | 1.543503 | 3 |
| Total | 1.39433E1 | 1.634977 | 9 |

Levene's Test of Equality of Error Variances[a]

Dependent Variable:result

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .057 | 2 | 6 | .945 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + cell

Tests of Between-Subjects Effects

Dependent Variable:result

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 4.216[a] | 2 | 2.108 | .737 | .517 |
| Intercept | 1749.749 | 1 | 1749.749 | 611.482 | .000 |
| cell | 4.216 | 2 | 2.108 | .737 | .517 |
| Error | 17.169 | 6 | 2.861 | | |
| Total | 1771.134 | 9 | | | |
| Corrected Total | 21.385 | 8 | | | |

a. R Squared = .197 (Adjusted R Squared = -.070)

Post Hoc Tests: Cell condition

Multiple Comparisons

| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | Cell | Cell+Neg. | -.92667 | 1.381180 | .788 | -5.16451 | 3.31117 |
| | | Cell+siRNA | -1.67333 | 1.381180 | .489 | -5.91117 | 2.56451 |
| | Cell+Neg. | Cell | .92667 | 1.381180 | .788 | -3.31117 | 5.16451 |
| | | Cell+siRNA | -.74667 | 1.381180 | .855 | -4.98451 | 3.49117 |
| | Cell+siRNA | Cell | 1.67333 | 1.381180 | .489 | -2.56451 | 5.91117 |
| | | Cell+Neg. | .74667 | 1.381180 | .855 | -3.49117 | 4.98451 |
| Scheffe | Cell | Cell+Neg. | -.92667 | 1.381180 | .805 | -5.35647 | 3.50314 |
| | | Cell+siRNA | -1.67333 | 1.381180 | .519 | -6.10314 | 2.75647 |
| | Cell+Neg. | Cell | .92667 | 1.381180 | .805 | -3.50314 | 5.35647 |
| | | Cell+siRNA | -.74667 | 1.381180 | .867 | -5.17647 | 3.68314 |
| | Cell+siRNA | Cell | 1.67333 | 1.381180 | .519 | -2.75647 | 6.10314 |
| | | Cell+Neg. | .74667 | 1.381180 | .867 | -3.68314 | 5.17647 |
| Dunnett t (2-sided)[a] | Cell | Cell+siRNA | -1.67333 | 1.381180 | .424 | -5.62731 | 2.28064 |
| | Cell+Neg. | Cell+siRNA | -.74667 | 1.381180 | .817 | -4.70064 | 3.20731 |

Based on observed means.

The error term is Mean Square(Error) = 2.861.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

## Homogeneous Subsets

**result**

|  | Cell condition | N | Subset |
|---|---|---|---|
|  |  |  | 1 |
| Tukey HSD[a] | Cell | 3 | 1.30767E1 |
|  | Cell+Neg. | 3 | 1.40033E1 |
|  | Cell+siRNA | 3 | 1.47500E1 |
|  | Sig. |  | .489 |
| Scheffe[a] | Cell | 3 | 1.30767E1 |
|  | Cell+Neg. | 3 | 1.40033E1 |
|  | Cell+siRNA | 3 | 1.47500E1 |
|  | Sig. |  | .519 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.861.

a. Uses Harmonic Mean Sample Size = 3.000.

## APPENDIX D Proliferation

ครั้งที่ 1

### Raw data

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.039 | 0.038 | 0.038 | 0.033 | 0.033 | 0.028 | 0.035 | 0.035 | 0.035 | 0.035 | 0.033 | 0.026 |
| B | 0.038 | 0.102 | 0.119 | 0.124 | 0.138 | 0.032 | 0.038 | 0.129 | 0.111 | 0.139 | 0.147 | 0.034 |
| C | 0.039 | 0.131 | 0.127 | 0.117 | 0.105 | 0.033 | 0.035 | 0.142 | 0.136 | 0.117 | 0.140 | 0.032 |
| D | 0.033 | 0.128 | 0.121 | 0.134 | 0.132 | 0.034 | 0.040 | 0.121 | 0.128 | 0.137 | 0.158 | 0.033 |
| E | 0.033 | 0.095 | 0.109 | 0.137 | 0.141 | 0.037 | 0.042 | 0.123 | 0.152 | 0.145 | 0.116 | 0.033 |
| F | 0.033 | 0.146 | 0.154 | 0.162 | 0.149 | 0.153 | 0.178 | 0.170 | 0.191 | 0.053 | 0.061 | 0.032 |
| G | 0.034 | 0.175 | 0.170 | 0.167 | 0.193 | 0.189 | 0.019 | 0.164 | 0.182 | 0.055 | 0.047 | 0.038 |
| H | 0.034 | 0.033 | 0.034 | 0.030 | 0.040 | 0.034 | 0.031 | 0.035 | 0.034 | 0.038 | 0.038 | 0.033 |

ครั้งที่ 2

### Raw data

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.036 | 0.037 | 0.037 | 0.034 | 0.033 | 0.032 | 0.035 | 0.034 | 0.027 | 0.034 | 0.034 | 0.041 |
| B | 0.026 | 0.114 | 0.115 | 0.123 | 0.129 | 0.035 | 0.035 | 0.121 | 0.130 | 0.129 | 0.143 | 0.040 |
| C | 0.035 | 0.122 | 0.119 | 0.127 | 0.124 | 0.034 | 0.036 | 0.136 | 0.148 | 0.127 | 0.115 | 0.032 |
| D | 0.035 | 0.138 | 0.148 | 0.155 | 0.150 | 0.035 | 0.041 | 0.157 | 0.143 | 0.168 | 0.170 | 0.035 |
| E | 0.041 | 0.131 | 0.136 | 0.127 | 0.133 | 0.033 | 0.042 | 0.132 | 0.139 | 0.154 | 0.165 | 0.034 |
| F | 0.027 | 0.149 | 0.159 | 0.147 | 0.158 | 0.174 | 0.188 | 0.187 | 0.171 | 0.052 | 0.059 | 0.034 |
| G | 0.034 | 0.147 | 0.166 | 0.160 | 0.174 | 0.194 | 0.215 | 0.175 | 0.181 | 0.047 | 0.044 | 0.037 |
| H | 0.038 | 0.033 | 0.035 | 0.032 | 0.033 | 0.041 | 0.038 | 0.037 | 0.034 | 0.028 | 0.038 | 0.034 |

ครั้งที่ 3

Raw data

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.035 | 0.027 | 0.027 | 0.034 | 0.039 | 0.028 | 0.029 | 0.035 | 0.036 | 0.028 | 0.033 | 0.034 |
| B | 0.034 | 0.155 | 0.136 | 0.143 | 0.173 | 0.036 | 0.032 | 0.146 | 0.139 | 0.125 | 0.162 | 0.030 |
| C | 0.029 | 0.172 | 0.174 | 0.187 | 0.185 | 0.037 | 0.031 | 0.143 | 0.189 | 0.183 | 0.197 | 0.035 |
| D | 0.031 | 0.186 | 0.178 | 0.197 | 0.169 | 0.029 | 0.039 | 0.191 | 0.163 | 0.186 | 0.202 | 0.029 |
| E | 0.033 | 0.176 | 0.181 | 0.169 | 0.192 | 0.033 | 0.034 | 0.186 | 0.174 | 0.195 | 0.158 | 0.036 |
| F | 0.029 | 0.187 | 0.158 | 0.216 | 0.192 | 0.198 | 0.224 | 0.154 | 0.196 | 0.045 | 0.053 | 0.035 |
| G | 0.027 | 0.159 | 0.164 | 0.202 | 0.178 | 0.237 | 0.206 | 0.235 | 0.217 | 0.056 | 0.061 | 0.031 |
| H | 0.031 | 0.046 | 0.346 | 0.038 | 0.037 | 0.034 | 0.038 | 0.030 | 0.032 | 0.034 | 0.036 | 0.026 |

B 2 -5 Cells, siRNA

C 2-5 Cells, siRNA + PHA

D 2-5 Cells, siRNA + IL-2

E 2-5 Cells, siRNA + Anti-TCR Ab

B 8-11 Cells, negative control siRNA

C 8-11 Cells, negative control siRNA + PMA

D 8-11 Cells, negative control siRNA + IL-2

E 8-11 Cells, negative control siRNA + Anti-TCR Ab

F 2-3, G 2-3 Cells

F 4-5, G 4-5 Cells + PHA

F 6-7, G 6-7 Cells + IL-2

F 8-9, G 8-9 Cells + Anti-TCR Ab

F 10-11, G 10-11 Cells without BrdU reagent

B 7, C 7, E 7, F 7 Medium

| Groups | | Cells | Cells+Neg. control | Cells+siRNA |
|---|---|---|---|---|
| Unstimulated | ครั้งที่ 1 | 0.117 | 0.098 | 0.087 |
| | ครั้งที่ 2 | 0.114 | 0.095 | 0.083 |
| | ครั้งที่ 3 | 0.133 | 0.114 | 0.112 |
| PHA | ครั้งที่ 1 | 0.115 | 0.099 | 0.085 |
| | ครั้งที่ 2 | 0.118 | 0.098 | 0.085 |
| | ครั้งที่ 3 | 0.158 | 0.155 | 0.147 |
| Anti-TCR Ab | ครั้งที่ 1 | 0.117 | 0.100 | 0.089 |
| | ครั้งที่ 2 | 0.123 | 0.114 | 0.095 |
| | ครั้งที่ 3 | 0.161 | 0.150 | 0.148 |
| IL-2 | ครั้งที่ 1 | 0.120 | 0.101 | 0.091 |
| | ครั้งที่ 2 | 0.144 | 0.126 | 0.112 |
| | ครั้งที่ 3 | 0.166 | 0.158 | 0.152 |

**Data Analysis**

The data were expressed as the mean with a standard deviation (SD). Statistical data were analyzed with SPSS ANOVA followed by Dunnett's comparison test. The significance criterion for the correlation measurements was set at 0.05

### 1. Unstimulated group

Univariate Analysis of Variance

Between-Subjects Factors

| | | Value Label | N |
|---|---|---|---|
| Cell condition | 1 | Cell only | 3 |
| | 2 | Neg. RNA | 3 |
| | 3 | siRNA | 3 |

Descriptive Statistics

Dependent Variable:result

| Cell condition | Mean | Std. Deviation | N |
|---|---|---|---|
| Cell only | .12133 | .010214 | 3 |
| Neg. RNA | .10233 | .010214 | 3 |
| siRNA | .09400 | .015716 | 3 |
| Total | .10589 | .016159 | 9 |

Levene's Test of Equality of Error Variances[a]

Dependent Variable:result

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .919 | 2 | 6 | .449 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + cell

Tests of Between-Subjects Effects

Dependent Variable:result

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .001[a] | 2 | .001 | 3.876 | .083 |
| Intercept | .101 | 1 | .101 | 664.381 | .000 |
| cell | .001 | 2 | .001 | 3.876 | .083 |
| Error | .001 | 6 | .000 | | |
| Total | .103 | 9 | | | |
| Corrected Total | .002 | 8 | | | |

a. R Squared = .564 (Adjusted R Squared = .418)

## Post Hoc Tests

### Cell condition

Multiple Comparisons

Dependent Variable:result

| | | | Mean Difference | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | (I) Cell condition | (J) Cell condition | (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| Tukey HSD | Cell only | Neg. RNA | .01900 | .010063 | .222 | -.01188 | .04988 |
| | | siRNA | .02733 | .010063 | .078 | -.00354 | .05821 |
| | Neg. RNA | Cell only | -.01900 | .010063 | .222 | -.04988 | .01188 |
| | | siRNA | .00833 | .010063 | .701 | -.02254 | .03921 |
| | siRNA | Cell only | -.02733 | .010063 | .078 | -.05821 | .00354 |
| | | Neg. RNA | -.00833 | .010063 | .701 | -.03921 | .02254 |
| Scheffe | Cell only | Neg. RNA | .01900 | .010063 | .247 | -.01327 | .05127 |
| | | siRNA | .02733 | .010063 | .090 | -.00494 | .05961 |
| | Neg. RNA | Cell only | -.01900 | .010063 | .247 | -.05127 | .01327 |
| | | siRNA | .00833 | .010063 | .723 | -.02394 | .04061 |
| | siRNA | Cell only | -.02733 | .010063 | .090 | -.05961 | .00494 |
| | | Neg. RNA | -.00833 | .010063 | .723 | -.04061 | .02394 |
| Dunnett t (2-sided)[a] | Cell only | siRNA | .02733 | .010063 | .060 | -.00147 | .05614 |
| | Neg. RNA | siRNA | .00833 | .010063 | .641 | -.02047 | .03714 |

Based on observed means.

The error term is Mean Square(Error) = .000.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

### Homogeneous Subsets

result

| | | | Subset |
|---|---|---|---|
| | Cell condition | N | 1 |
| Tukey HSD[a] | siRNA | 3 | .09400 |
| | Neg. RNA | 3 | .10233 |
| | Cell only | 3 | .12133 |
| | Sig. | | .078 |

| | | | |
|---|---|---|---|
| Scheffe[a] | siRNA | 3 | .09400 |
| | Neg. RNA | 3 | .10233 |
| | Cell only | 3 | .12133 |
| | Sig. | | .090 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .000.

a. Uses Harmonic Mean Sample Size = 3.000.

Estimated Marginal Means

Cell condition

Dependent Variable:result

| Cell condition | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Cell only | .121 | .007 | .104 | .139 |
| Neg. RNA | .102 | .007 | .085 | .120 |
| siRNA | .094 | .007 | .077 | .111 |

## 2. PHA stimulated group

### Univariate Analysis of Variance

**Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| Cell condition | 1 | Cell only | 3 |
| | 2 | Neg. RNA | 3 |
| | 3 | siRNA | 4 |

Descriptive Statistics

Dependent Variable:result

| Cell condition | Mean | Std. Deviation | N |
|---|---|---|---|
| Cell only | .13033 | .024007 | 3 |
| Neg. RNA | .11733 | .022426 | 3 |
| siRNA | .10625 | .035087 | 4 |
| Total | .12080 | .029020 | 10 |

Levene's Test of Equality of Error Variances[a]

Dependent Variable:result

| F | df1 | df2 | Sig. |
|---|---|---|---|
| 2.671 | 2 | 7 | .137 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + cell

Tests of Between-Subjects Effects

Dependent Variable:result

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .000[a] | 2 | .000 | .191 | .831 |
| Intercept | .144 | 1 | .144 | 140.695 | .000 |
| cell | .000 | 2 | .000 | .191 | .831 |
| Error | .007 | 7 | .001 | | |
| Total | .154 | 10 | | | |
| Corrected Total | .008 | 9 | | | |

a. R Squared = .052 (Adjusted R Squared = -.219)

Post Hoc Tests

Cell condition

Multiple Comparisons

Dependent Variable:result

| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | Cell only | Neg. RNA | .01300 | .026164 | .875 | -.06406 | .09006 |
| | | siRNA | .01408 | .024475 | .837 | -.05800 | .08616 |
| | Neg. RNA | Cell only | -.01300 | .026164 | .875 | -.09006 | .06406 |
| | | siRNA | .00108 | .024475 | .999 | -.07100 | .07316 |
| | siRNA | Cell only | -.01408 | .024475 | .837 | -.08616 | .05800 |
| | | Neg. RNA | -.00108 | .024475 | .999 | -.07316 | .07100 |
| Scheffe | Cell only | Neg. RNA | .01300 | .026164 | .886 | -.06754 | .09354 |
| | | siRNA | .01408 | .024475 | .851 | -.06125 | .08942 |
| | Neg. RNA | Cell only | -.01300 | .026164 | .886 | -.09354 | .06754 |
| | | siRNA | .00108 | .024475 | .999 | -.07425 | .07642 |
| | siRNA | Cell only | -.01408 | .024475 | .851 | -.08942 | .06125 |
| | | Neg. RNA | -.00108 | .024475 | .999 | -.07642 | .07425 |
| Dunnett t (2-sided)[a] | Cell only | siRNA | .01408 | .024475 | .801 | -.05360 | .08177 |
| | Neg. RNA | siRNA | .00108 | .024475 | .999 | -.06660 | .06877 |

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

## Homogeneous Subsets

result

| | Cell condition | N | Subset |
|---|---|---|---|
| | | | 1 |
| Tukey HSD[a] | siRNA | 4 | .10625 |
| | Neg. RNA | 3 | .11733 |
| | Cell only | 3 | .13033 |
| | Sig. | | .844 |
| Scheffe[a] | siRNA | 4 | .10625 |
| | Neg. RNA | 3 | .11733 |
| | Cell only | 3 | .13033 |
| | Sig. | | .857 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.273.

## Estimated Marginal Means

Cell condition

Dependent Variable:result

| Cell condition | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Cell only | .130 | .019 | .087 | .174 |
| Neg. RNA | .117 | .019 | .074 | .161 |
| siRNA | .106 | .016 | .078 | .154 |

## 3. Anti-TCR Ab stimulated group

### Univariate Analysis of Variance

**Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| Cell condition | 1 | Cell only | 3 |
| | 2 | Neg. RNA | 3 |
| | 3 | siRNA | 3 |

Descriptive Statistics

Dependent Variable:result

| Cell condition | Mean | Std. Deviation | N |
|---|---|---|---|
| Cell only | .13367 | .023861 | 3 |
| Neg. RNA | .12133 | .025794 | 3 |
| siRNA | .11067 | .032470 | 3 |
| Total | .12189 | .025915 | 9 |

Levene's Test of Equality of Error Variances[a]

Dependent Variable:result

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .376 | 2 | 6 | .702 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + cell

### Tests of Between-Subjects Effects

Dependent Variable:result

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .001[a] | 2 | .000 | .521 | .619 |
| Intercept | .134 | 1 | .134 | 175.245 | .000 |
| cell | .001 | 2 | .000 | .521 | .619 |
| Error | .005 | 6 | .001 | | |
| Total | .139 | 9 | | | |
| Corrected Total | .005 | 8 | | | |

a. R Squared = .148 (Adjusted R Squared = -.136)

## Post Hoc Tests: Cell condition

Multiple Comparisons

| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | Cell only | Neg. RNA | .01233 | .022554 | .852 | -.05687 | .08153 |
| | | siRNA | .02300 | .022554 | .592 | -.04620 | .09220 |
| | Neg. RNA | Cell only | -.01233 | .022554 | .852 | -.08153 | .05687 |
| | | siRNA | .01067 | .022554 | .886 | -.05853 | .07987 |
| | siRNA | Cell only | -.02300 | .022554 | .592 | -.09220 | .04620 |
| | | Neg. RNA | -.01067 | .022554 | .886 | -.07987 | .05853 |
| Scheffe | Cell only | Neg. RNA | .01233 | .022554 | .864 | -.06000 | .08467 |
| | | siRNA | .02300 | .022554 | .619 | -.04934 | .09534 |
| | Neg. RNA | Cell only | -.01233 | .022554 | .864 | -.08467 | .06000 |
| | | siRNA | .01067 | .022554 | .896 | -.06167 | .08300 |
| | siRNA | Cell only | -.02300 | .022554 | .619 | -.09534 | .04934 |
| | | Neg. RNA | -.01067 | .022554 | .896 | -.08300 | .06167 |
| Dunnett t (2-sided)[a] | Cell only | siRNA | .02300 | .022554 | .527 | -.04157 | .08757 |
| | Neg. RNA | siRNA | .01067 | .022554 | .855 | -.05390 | .07523 |

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

## Homogeneous Subsets

result

| | Cell condition | N | Subset |
|---|---|---|---|
| | | | 1 |
| Tukey HSD[a] | siRNA | 3 | .11067 |
| | Neg. RNA | 3 | .12133 |
| | Cell only | 3 | .13367 |
| | Sig. | | .592 |
| Scheffe[a] | siRNA | 3 | .11067 |
| | Neg. RNA | 3 | .12133 |
| | Cell only | 3 | .13367 |
| | Sig. | | .619 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

## Estimated Marginal Means

**Cell condition**

Dependent Variable:result

| Cell condition | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Cell only | .134 | .016 | .095 | .173 |
| Neg. RNA | .121 | .016 | .082 | .160 |
| siRNA | .111 | .016 | .072 | .150 |

## 4. IL-2 stimuated group

### Univariate Analysis of Variance

Between-Subjects Factors

| | | Value Label | N |
|---|---|---|---|
| Cell condition | 1 | Cell only | 3 |
| | 2 | Neg. RNA | 3 |
| | 3 | siRNA | 3 |

Descriptive Statistics

Dependent Variable:result

| Cell condition | Mean | Std. Deviation | N |
|---|---|---|---|
| Cell only | .14333 | .023007 | 3 |
| Neg. RNA | .12833 | .028572 | 3 |
| siRNA | .11833 | .030989 | 3 |
| Total | .13000 | .026368 | 9 |

Levene's Test of Equality of Error Variances[a]

Dependent Variable:result

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .181 | 2 | 6 | .839 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + cell

Tests of Between-Subjects Effects

Dependent Variable:result

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .001[a] | 2 | .000 | .618 | .570 |
| Intercept | .152 | 1 | .152 | 197.875 | .000 |
| cell | .001 | 2 | .000 | .618 | .570 |
| Error | .005 | 6 | .001 | | |
| Total | .158 | 9 | | | |
| Corrected Total | .006 | 8 | | | |

a. R Squared = .171 (Adjusted R Squared = -.106)

## Post Hoc Tests: Cell condition

Multiple Comparisons

| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | Cell only | Neg. RNA | .01500 | .022637 | .793 | -.05446 | .08446 |
| | | siRNA | .02500 | .022637 | .546 | -.04446 | .09446 |
| | Neg. RNA | Cell only | -.01500 | .022637 | .793 | -.08446 | .05446 |
| | | siRNA | .01000 | .022637 | .900 | -.05946 | .07946 |
| | siRNA | Cell only | -.02500 | .022637 | .546 | -.09446 | .04446 |
| | | Neg. RNA | -.01000 | .022637 | .900 | -.07946 | .05946 |
| Scheffe | Cell only | Neg. RNA | .01500 | .022637 | .809 | -.05760 | .08760 |
| | | siRNA | .02500 | .022637 | .574 | -.04760 | .09760 |
| | Neg. RNA | Cell only | -.01500 | .022637 | .809 | -.08760 | .05760 |
| | | siRNA | .01000 | .022637 | .908 | -.06260 | .08260 |
| | siRNA | Cell only | -.02500 | .022637 | .574 | -.09760 | .04760 |
| | | Neg. RNA | -.01000 | .022637 | .908 | -.08260 | .06260 |
| Dunnett t (2-sided)[a] | Cell only | siRNA | .02500 | .022637 | .480 | -.03980 | .08980 |
| | Neg. RNA | siRNA | .01000 | .022637 | .871 | -.05480 | .07480 |

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

**result**

| | | | Subset |
|---|---|---|---|
| | Cell condition | N | 1 |
| Tukey HSD[a] | siRNA | 3 | .11833 |
| | Neg. RNA | 3 | .12833 |
| | Cell only | 3 | .14333 |
| | Sig. | | .546 |
| Scheffe[a] | siRNA | 3 | .11833 |
| | Neg. RNA | 3 | .12833 |
| | Cell only | 3 | .14333 |
| | Sig. | | .574 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

Estimated Marginal Means

Cell condition

Dependent Variable:result

| | | | 95% Confidence Interval | |
|---|---|---|---|---|
| Cell condition | Mean | Std. Error | Lower Bound | Upper Bound |
| Cell only | .143 | .016 | .104 | .183 |
| Neg. RNA | .128 | .016 | .089 | .168 |
| siRNA | .118 | .016 | .079 | .158 |

## APPENDIX E IL-2

| Groups | | Cells | Cells+Neg. control | Cells+siRNA |
|---|---|---|---|---|
| Unstimulated | ครั้งที่ 1 | 0 | 0 | 0 |
| | ครั้งที่ 2 | 0 | 0 | 0 |
| | ครั้งที่ 3 | 0 | 0 | 0 |
| PHA + PMA | ครั้งที่ 1 | 440.822 | 357.611 | 322.374 |
| | ครั้งที่ 2 | 442.975 | 374.281 | 374.615 |
| | ครั้งที่ 3 | 455.252 | 359.945 | 341.059 |
| CD3 +CD28 | ครั้งที่ 1 | 21.703 | 21.624 | 4.655 |
| | ครั้งที่ 2 | 26.081 | 13.856 | 2.809 |
| | ครั้งที่ 3 | 39.139 | 24.324 | 5.239 |

## Data Analysis

The data were expressed as the mean with a standard deviation (SD). Statistical data were analyzed with SPSS One-way ANOVA followed by Dunnett's comparison test. The significance criterion for the correlation measurements was set at 0.05

### 1. PHA/PMA stimulated group

Oneway Analysis of Variance

**Descriptives**

result

|  |  |  |  |  | 95% Confidence Interval for Mean |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound | Minimum | Maximum |
| Cell only | 3 | 4.46350E2 | 7.784440 | 4.494349 | 427.01205 | 465.68729 | 440.822 | 455.252 |
| siRNA | 3 | 3.46016E2 | 26.470916 | 1.528299E1 | 280.25860 | 411.77340 | 322.374 | 374.615 |
| negRNA | 3 | 3.63946E2 | 9.026418 | 5.211405 | 341.52280 | 386.36853 | 357.611 | 374.281 |
| Total | 9 | 3.85437E2 | 48.559632 | 1.618654E1 | 348.11087 | 422.76335 | 322.374 | 455.252 |

Test of Homogeneity of Variances

result

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 2.781 | 2 | 6 | .140 |

ANOVA

result

|  | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 17178.737 | 2 | 8589.368 | 30.575 | .001 |
| Within Groups | 1685.566 | 6 | 280.928 |  |  |
| Total | 18864.303 | 8 |  |  |  |

Post Hoc Tests

### Multiple Comparisons

Dependent Variable:result

| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | Cell only | siRNA | 100.333667* | 1.368522E1 | .001 | 58.34367 | 142.32367 |
| | | negRNA | 82.404000* | 1.368522E1 | .002 | 40.41400 | 124.39400 |
| | siRNA | Cell only | -100.333667* | 1.368522E1 | .001 | -142.32367 | -58.34367 |
| | | negRNA | -17.929667 | 1.368522E1 | .440 | -59.91967 | 24.06033 |
| | negRNA | Cell only | -82.404000* | 1.368522E1 | .002 | -124.39400 | -40.41400 |
| | | siRNA | 17.929667 | 1.368522E1 | .440 | -24.06033 | 59.91967 |
| Scheffe | Cell only | siRNA | 100.333667* | 1.368522E1 | .001 | 56.44164 | 144.22569 |
| | | negRNA | 82.404000* | 1.368522E1 | .003 | 38.51198 | 126.29602 |
| | siRNA | Cell only | -100.333667* | 1.368522E1 | .001 | -144.22569 | -56.44164 |
| | | negRNA | -17.929667 | 1.368522E1 | .470 | -61.82169 | 25.96236 |
| | negRNA | Cell only | -82.404000* | 1.368522E1 | .003 | -126.29602 | -38.51198 |
| | | siRNA | 17.929667 | 1.368522E1 | .470 | -25.96236 | 61.82169 |
| Dunnett t (2-sided)[a] | Cell only | negRNA | 82.404000* | 1.368522E1 | .002 | 43.22664 | 121.58136 |
| | siRNA | negRNA | -17.929667 | 1.368522E1 | .377 | -57.10703 | 21.24769 |

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

result

|  | Cell condition | N | Subset for alpha = 0.05 |  |
|---|---|---|---|---|
|  |  |  | 1 | 2 |
| Tukey B$^a$ | siRNA | 3 | 3.46016E2 |  |
|  | negRNA | 3 | 3.63946E2 |  |
|  | Cell only | 3 |  | 4.46350E2 |
| Scheffe$^a$ | siRNA | 3 | 3.46016E2 |  |
|  | negRNA | 3 | 3.63946E2 |  |
|  | Cell only | 3 |  | 4.46350E2 |
|  | Sig. |  | .470 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

## 2. CD3/CD28 stimulated group

### Oneway

**Descriptives**

result

|  | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |  | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | Lower Bound | Upper Bound |  |  |
| Cell only | 3 | 2.89743E1 | 9.070946 | 5.237113 | 6.44085 | 51.50781 | 21.703 | 39.139 |
| siRNA | 3 | 4.23433 | 1.268442 | .732335 | 1.08335 | 7.38532 | 2.809 | 5.239 |
| negRNA | 3 | 2.01347E1 | 5.099798 | 2.944370 | 7.46607 | 32.80327 | 14.456 | 24.324 |
| Total | 9 | 1.77811E1 | 12.056253 | 4.018751 | 8.51386 | 27.04837 | 2.809 | 39.139 |

**Test of Homogeneity of Variances**

result

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 4.205 | 2 | 6 | .072 |

### ANOVA

result

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 943.028 | 2 | 471.514 | 12.871 | .007 |
| Within Groups | 219.798 | 6 | 36.633 | | |
| Total | 1162.826 | 8 | | | |

## Post Hoc Tests

### Multiple Comparisons

Dependent Variable:result

| | (I) Cell condition | (J) Cell condition | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | Cell only | siRNA | 24.740000* | 4.941861 | .006 | 9.57701 | 39.90299 |
| | | negRNA | 8.839667 | 4.941861 | .251 | -6.32332 | 24.00265 |
| | siRNA | Cell only | -24.740000* | 4.941861 | .006 | -39.90299 | -9.57701 |
| | | negRNA | -15.900333* | 4.941861 | .042 | -31.06332 | -.73735 |
| | negRNA | Cell only | -8.839667 | 4.941861 | .251 | -24.00265 | 6.32332 |
| | | siRNA | 15.900333* | 4.941861 | .042 | .73735 | 31.06332 |
| Scheffe | Cell only | siRNA | 24.740000* | 4.941861 | .007 | 8.89018 | 40.58982 |
| | | negRNA | 8.839667 | 4.941861 | .277 | -7.01016 | 24.68949 |
| | siRNA | Cell only | -24.740000* | 4.941861 | .007 | -40.58982 | -8.89018 |
| | | negRNA | -15.900333* | 4.941861 | .049 | -31.75016 | -.05051 |
| | negRNA | Cell only | -8.839667 | 4.941861 | .277 | -24.68949 | 7.01016 |
| | | siRNA | 15.900333* | 4.941861 | .049 | .05051 | 31.75016 |
| Dunnett t (2-sided)[a] | Cell only | negRNA | 8.839667 | 4.941861 | .206 | -5.30765 | 22.98698 |
| | siRNA | negRNA | -15.900333* | 4.941861 | .032 | -30.04765 | -1.75302 |

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

## Homogeneous Subsets

**result**

| | Cell condition | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD[a] | siRNA | 3 | 4.23433 | |
| | negRNA | 3 | | 20.13467 |
| | Cell only | 3 | | 28.97433 |
| | Sig. | | 1.000 | .251 |
| Tukey B[a] | siRNA | 3 | 4.23433 | |
| | negRNA | 3 | | 20.13467 |
| | Cell only | 3 | | 28.97433 |
| Scheffe[a] | siRNA | 3 | 4.23433 | |
| | negRNA | 3 | | 20.13467 |
| | Cell only | 3 | | 28.97433 |
| | Sig. | | 1.000 | .277 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

# BIOGRAPHY

# BIOGRAPHY

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| | |
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