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DESIGN OF EXPERIMENT (DoE) METHODOLOGY EFFECTIVELY REDUCES COST & TIME FOR ANALYTICAL METHOD DEVELOPMENT

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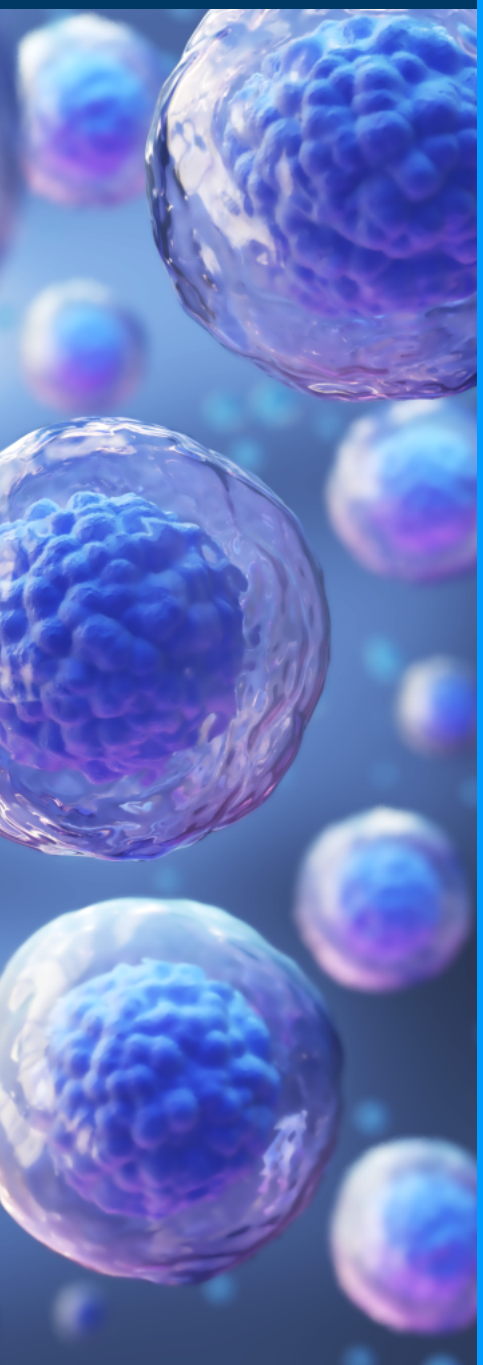

Abstract

Robust assays are a cornerstone of cell therapy program success, inclusive of process development through commercialization and ultimately supportive of product release to the patient population. Thus, assay development underpins a successful cell therapy program as it enables accurate and precise assessment of critical process parameters, ensuring therapeutic product consistency and effectiveness. Assay development, especially for complex cell-based assays, can represent a potentially prohibitive investment of time and money, especially for innovator companies focused on early phase programs. To help address this issue, Kincell Bio has adopted a Design of Experiment (DoE) methodology to optimize complex cell-based assays. This approach accelerates method development by allowing for assessment of more parameters in a single study than traditional one factor at a time experimentation. Moreover, DoEs facilitate a more resource-efficient use of experimental resources and precious raw materials, which ultimately reduces the overall cost of development. Through practical examples using DoE methodology to develop and optimize an IFN-γ release assay and flow cytometry based proliferation assay, we demonstrate how DoE techniques can lead to significant time savings, cost reductions, and improved decision-making in the development of robust analytical methods for cell therapy programs.

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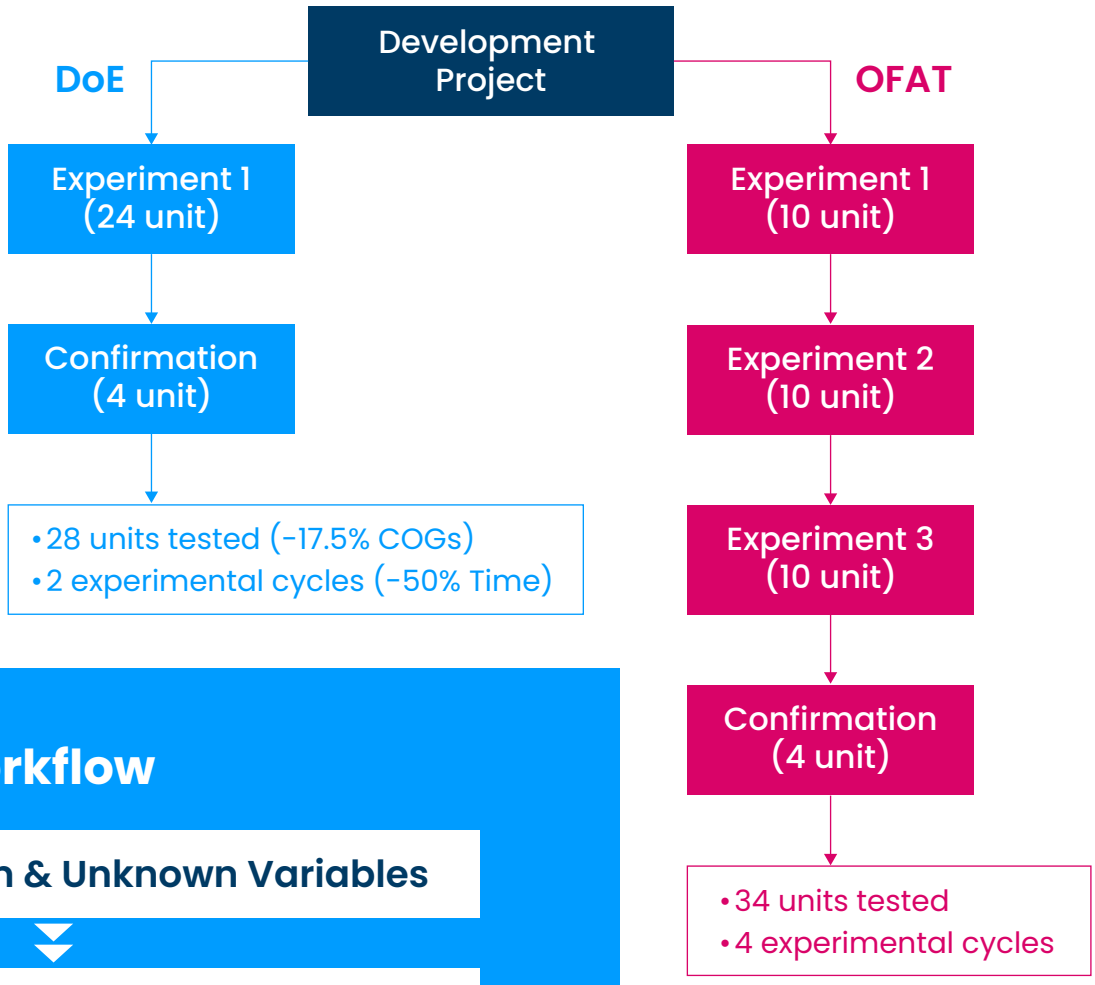
Graphical Abstract



Kincell DoE Workflow

1. Identify Problem & Unknown Variables

2. Meeting between DoE and Topical SME

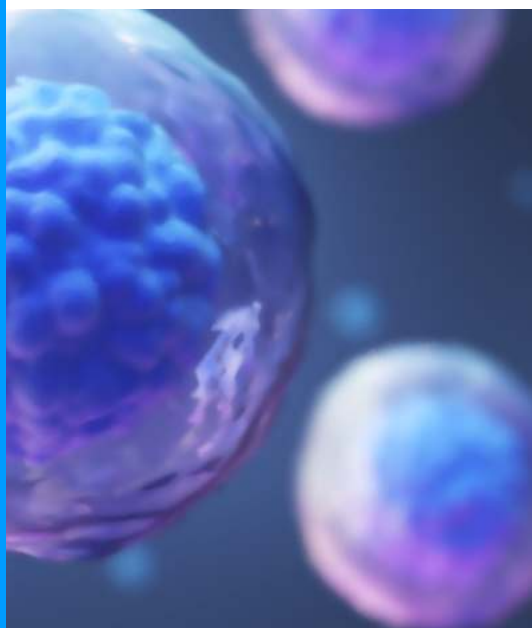
Identify:

- Responses
- Factors
- Factor Interactions and Power

3. Plan and execute DoE

4. Analyze data

- Involves a consideration of both the DoE output and SME expertise



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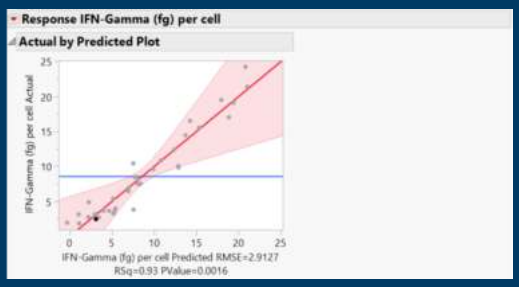
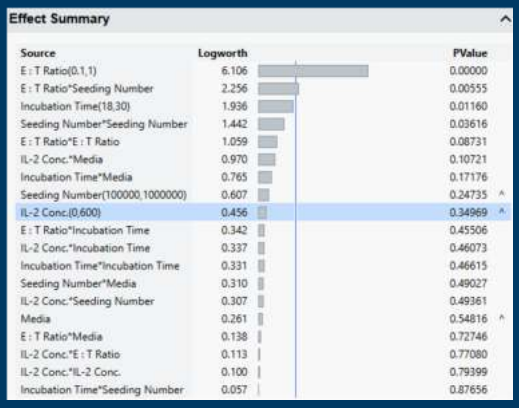
Example 1: IFN-γ Secretion Assay

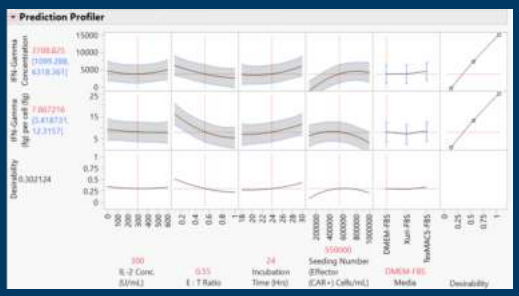
- A 36-unit DoE was performed to optimize an ELISA-based IFN-γ release assay.
- The Kincell DoE workflow was followed and responses, factors, and interactions identified to ensure an efficient study inclusive of all needed parameters.
- Study proved to be predictive (P value of 0.0016) and R2 values of 0.93 **(A)**
- Effect summary of the DoE identified that media type, seeding number, and IL-2 concentration did not have a significant impact on IFN-γ production ($p>0.05$), meanwhile E:T ratio and incubation time did have a significant impact on IFN-γ production ($p<0.05$) **(B)**.
- A follow-up experiment evaluating two DP lots over a 72-hour period using DoE determined parameters was performed to set and incubation time range and E:T ratio for the assay.
- Using the Prediction Profiler **(C)** and Effect Summary, Xuri-FBS media was used, no IL-2, and 5E+05 effector cells/mL were seeded for this follow-up experiment.
- Screening of these samples showed that an 0.5 E:T displayed gradual increase of IFN-γ between 18 and 42-hours of incubation, meanwhile an E:T of 0.1 was at its maximal IFN-γ output beginning at the first time point, a result consistent with the lower ratio **(D)**.
- Additional replicates for 0.5 E:T ratios between the two lots exhibited similar levels of IFN-γ production at 18-hours, however, by 42 hours IFN-γ production between the two lots plateaued and differences between the two lots were observed **(E)**.
- Kincell Bio SME evaluated results of this follow-up experiment and with the DoE output determined the final assay conditions described in **(F)**.
- This workflow led to a statistically optimized assay consistent with existing knowledge in the field with data supporting selection of all variables.



A



B



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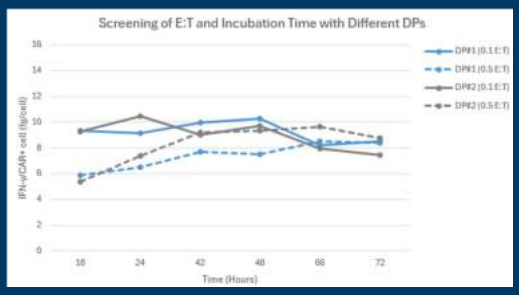
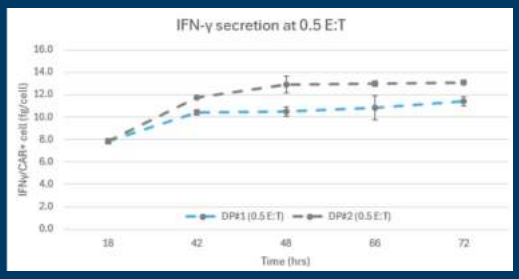


D



E

SME Adjusted Parameters

IL2 Conc. (IU/mL)	E:T Ratio	Incubation Time (Hrs)	Seeding Number (Effector CAR+ Cells/mL)	Media
0	0.5	48±2	5E+05	Xuri+5% FBS

F

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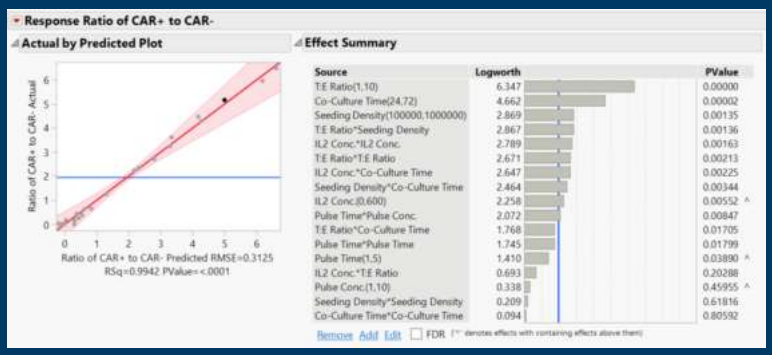
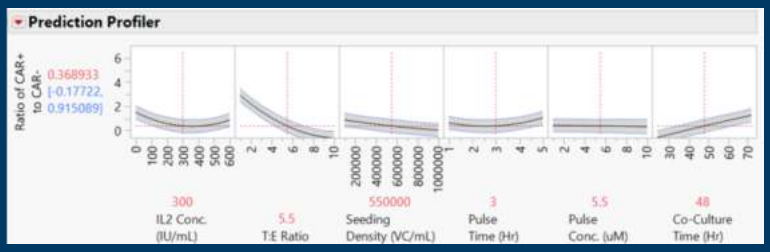
Example 2: Flow Cytometry Based Cell Proliferation Assay

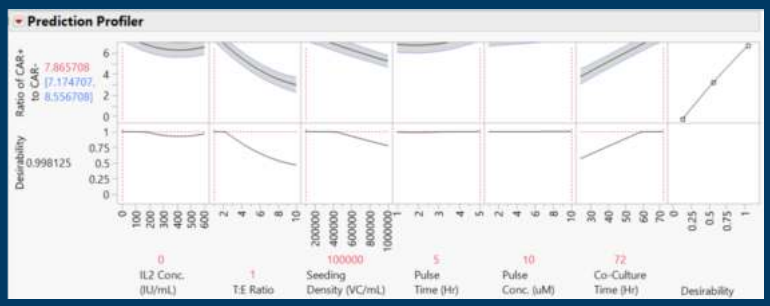
- A 24-unit DoE was performed to optimize a flow cytometry-based cell proliferation assay.
- The Kincell DoE workflow was followed and responses, factors, and interactions identified to ensure an efficient study inclusive of all needed parameters.
- Study proved to be predictive (P value < 0.0001) and an R2 value of 0.9942 **(A)**
- Results were assessed and the optimized using the JMP optimize desirability function **(B and C)**
- Kincell Bio SME proceeded to evaluate the optimized results and modify them consistent with the literature and Kincell Bio experience as described below:
  - IL2 conc.: 0
  - T:E Ratio: 1
  - Seeding Density: 2E+05
  - Pulse Time: 3h
  - Pulse Conc.: 10µM
  - Co-Culture Time: 72h
- This workflow led to a statistically optimized assay consistent with existing knowledge in the field with data supporting selection of all variables.



A



B



C

SME Adjusted Parameters

IL2 Conc. (IU/mL)	T:E Ratio	Seeding Density (VC/mL)	Pulse Time (Hr)	Pulse Conc. (µM)	Co-Culture Time (Hr)
0	1	2E+05	3	10	72

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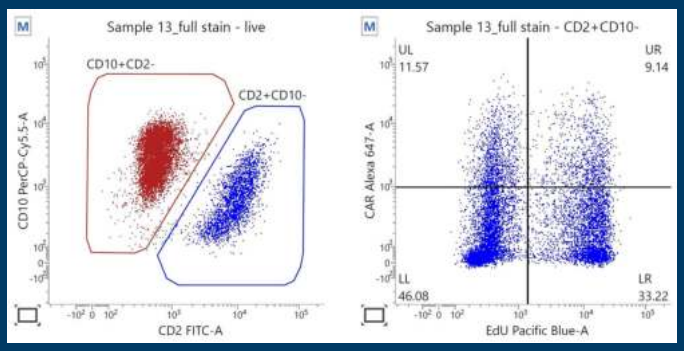
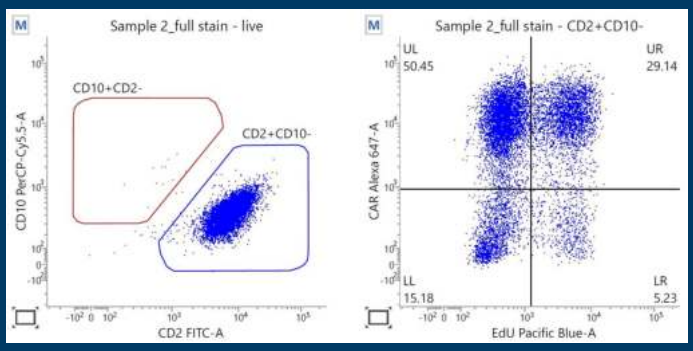
Example 2: Flow Cytometry Based Cell Proliferation Assay

SME Interpretation and Revisions (examples)

CAR down reg due to persistent target engagement



Reduced population resolution due to pulse time & conc

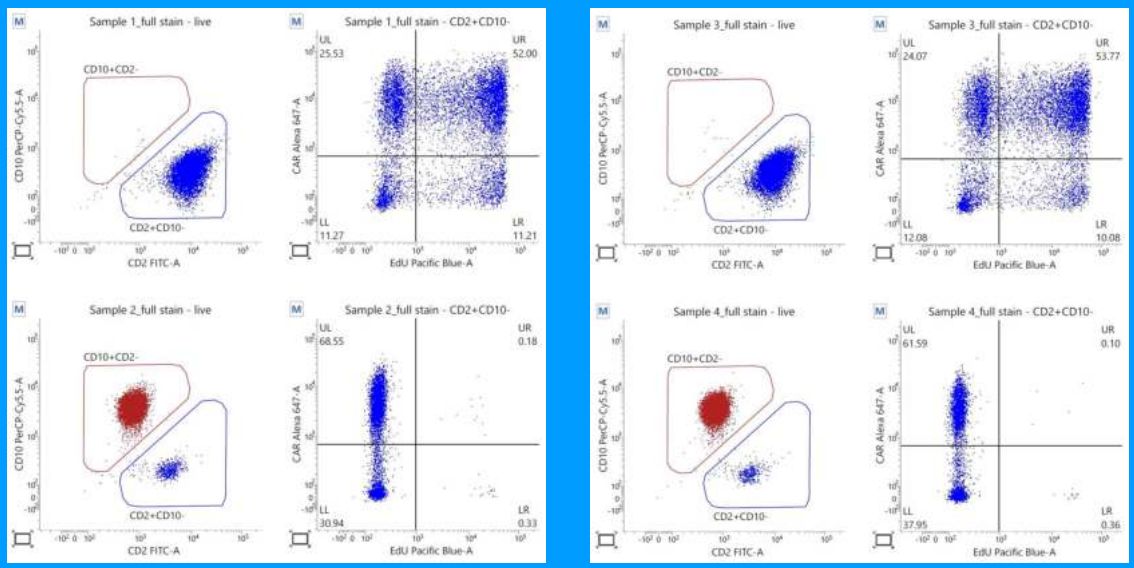


Final Assay: DP (Lot A)

Final Assay: DP (Lot B)

DP + Target

DP + Target (CD19 KO)



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Discussion

Both cost and time are critical components that can define a programs success. Therefore, approaches to reducing both time and cost while delivering an equally robust assay will provide a meaningful impact. Using the DoE approach, with SME guidance, allows rapid and efficient progression in the early stages of assay development while providing valuable information for the drug product being examined.

Acknowledgments

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