Streamlining Potency Testing of Immune Effector Cells Using Simple Plex Assays

Introduction

Potency testing of gene-modified cell therapies is a critical requirement for the release of CAR-T cell products. While validated potency assays are required at the time of product licensing, their importance extends across preclinical development, manufacturing, dosing, comparability, and stability studies. Early development and qualification of these assays are essential to ensure consistent product performance and clinical success.

Regulatory guidance encourages a matrixed strategy that includes multiple orthogonal assays to assess potency[1]. As clinical data accumulates, the panel of assays may be refined. Among current FDA-approved CAR-T therapies, cytokine secretion—particularly interferon-gamma (IFN-γ) release—remains a primary measure of biological activity. IFN-γ secretion correlates with anti-tumor response following target cell engagement and is commonly assessed alongside cell viability and other product-specific metrics.

The Simple Plex™ Platform can be used to measure IFN-γ secretion as a rapid, precise, and scalable readout of CAR-T cell potency, all on an automated instrument that is 21 CFR Part 11 compliant. Using co-culture models with Nalm-6 and K562 target cells, we show that IFN-γ secretion correlates with antigen-specific cytotoxicity and aligns with orthogonal potency assays. We also highlight the flexibility of Simple Plex Platform to detect additional cytokines, enabling a broader characterization of immune effector function.

Streamlined workflow with Simple Plex

With automated workflows, minimal hands-on time, and regulatory-ready performance, the **Simple Plex Platform** offers a powerful solution for accelerating potency testing in cell therapy development and manufacturing. The streamlined assay workflow gives researchers high-quality potency data in just 90 minutes with minimal hands-on time—ideal for supporting larger patient numbers in cell therapy manufacturing environments.

Unlike flow- or luciferase-based cytotoxicity assays that only measure target cell responses, the Simple Plex IFN-gamma assay directly quantifies effector cytokine production (see Figure 1). Plus, Simple Plex assays run on the Ella™ System—a benchtop automated ELISA Platform that delivers accurate, reproducible data without manual steps. The result: faster turnaround than flow-based methods and no need for tumor cell line editing required by luciferase-based assays. The Simple Plex IFN-gamma assay features a unique microfluidic cartridge design that reduces user error and ensures consistent performance across labs, instruments, and operators.

FIGURE // 01

Measuring CAR T & CAR NK Cell Functionality

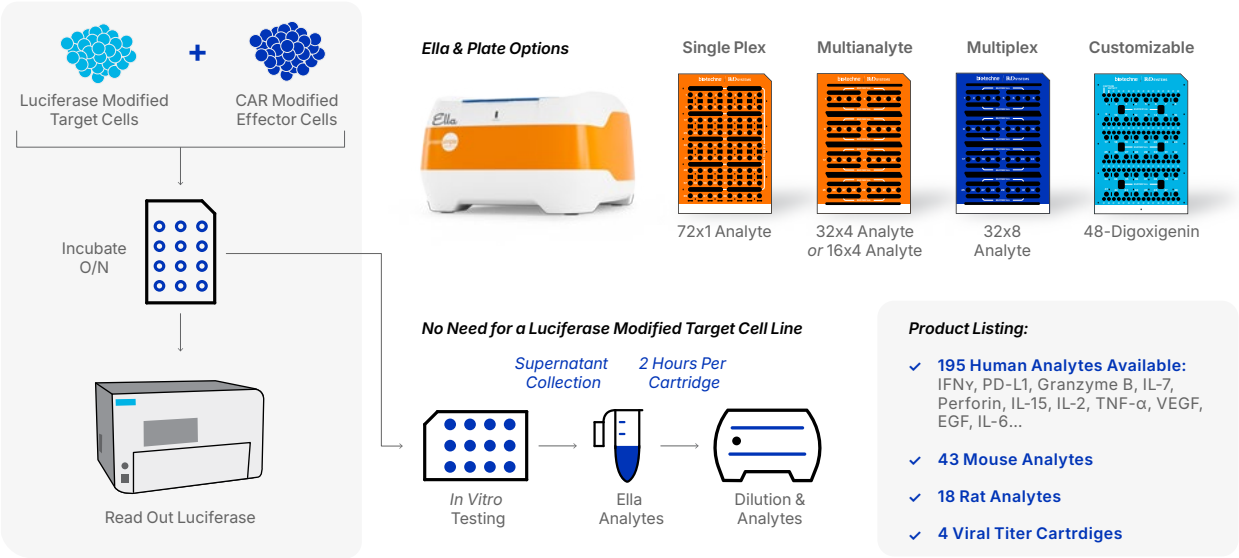

Figure 1. Simple Plex IFN-γ assays directly measure cytokine release from effector cells—eliminating the need for engineered target cell lines.

Demonstrating Assay Precision

FDA recommends that potency assays should be precise, accurate, specific, and robust[2]. These criteria ensure the assay can reliably measure the biological activity of a cell therapy product and demonstrate consistent performance throughout manufacturing and release. Simple Plex assays are validated to meet FDA expectations for precision, accuracy, specificity, and robustness. The Simple Plex IFN-γ assay features a broad dynamic range (0.17–4000 pg/mL), an LOD of 0.05 pg/mL, and fully automated protocols on a 21 CFR Part 11–compliant platform to minimize user variability and enable cross-site reproducibility.

For this experiment, the precision of the Simple Plex IFN-gamma assay was determined using high and low concentrations of recombinant human IFN-gamma in sample diluent. For intra-assay precision, each control was tested 16 times in a single assay. For inter-assay precision, replicates of each control were tested in multiple assays performed by at least three technicians using two lots of reagents. Intra- and inter-assay precision are summarized in Table 1.

Precision

Intra-Assay Precision: Each control was tested 16 times in one assay.

Inter-Assay Precision: Replicates of each control were tested in multiple assays performed by at least three technicians using two lots of reagents.

TABLE // 01

Simple Plex Precision

Parameter	Intra-Assay		Inter-Assay	
Sample	**Low**	**High**	**Low**	**High**
n	16	16	38	39
Mean (pg/mL)	21.1	1175	23.4	1192
Standard Deviation	1.48	33.4	2.52	99.4
CV (%)	7.0	2.8	10.8	8.3

Table 1. Simple Plex IFN-gamma intra-assay and inter-assay precision.

A 12-point standard curve prepared using 8 replicates of each standard concentration also demonstrated high precision across the broad dynamic range of the assay (0.17 - 4000 pg/mL). The mean coefficient of variation between replicates was 5.8% (Figure 2).

FIGURE // 02

Simple Plex Precision

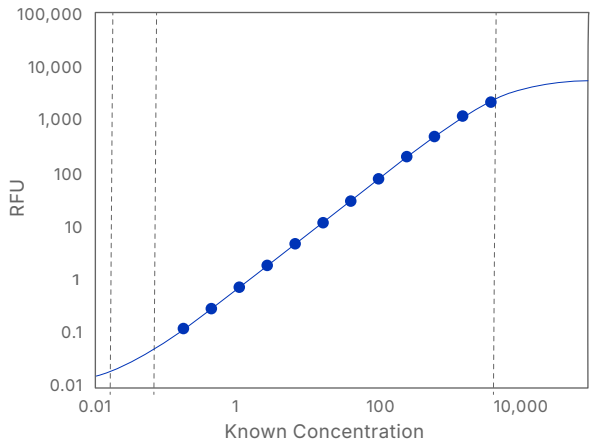

Figure 2. Simple Plex IFN-gamma standard curve. Points indicate the mean RFU for each standard concentration (n=8). Error bars indicate the maximum and minimum RFU at each standard concentration.

Quantitative Measurement of CAR-T Cell Potency

The **Simple Plex IFN-γ assay** provides quantitative insights into CAR-T activity across a variety of donors and effector-to-target (E:T) ratios. In co-culture experiments with Nalm-6 cells, CAR-T cells consistently secreted IFN-γ, while co-cultures with antigen-negative K562 cells produced minimal response—demonstrating both specificity and dynamic range of the assay (see Figure 3).

FIGURE // 03

Simple Plex Assay Measure

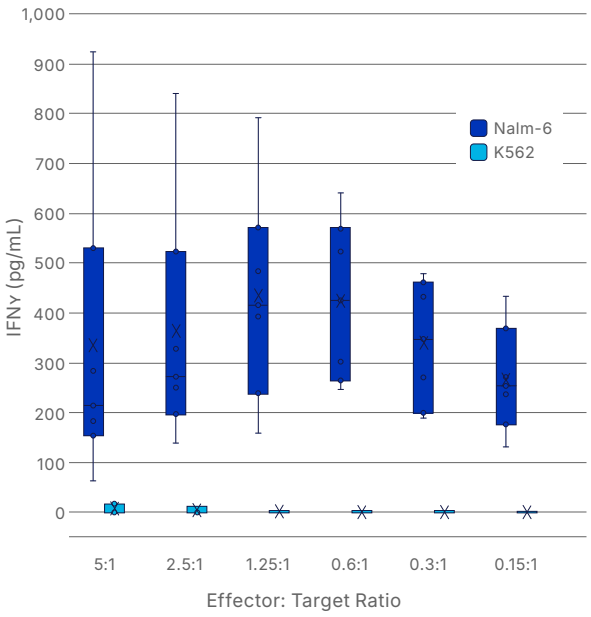

Figure 3. The Simple Plex assays measure IFNγ release when anti-CD19 CAR-T cells are co-cultured with Nalm-6 cells (dark blue, n = 7 donors, measured in duplicate or triplicate across varying E:T ratios. Minimal IFNγ release is observed when anti-CD19 CAR-T cells are co-cultured with K562 cells (light blue, n = 5 donors).

IFN-γ Secretion Aligns with Orthogonal Potency Readouts

To further validate the utility of IFN-γ secretion as a potency assay, we compared results with a luciferase-based cytolysis assay. CAR-T cells exhibited selective cytotoxicity against Nalm-6-luciferase targets, mirroring the IFN-γ secretion profile. (see Figure 4). These orthogonal approaches confirm the activation and specificity of the CAR-modified cells.

FIGURE // 04

Cell Lysis & IFNγ Release

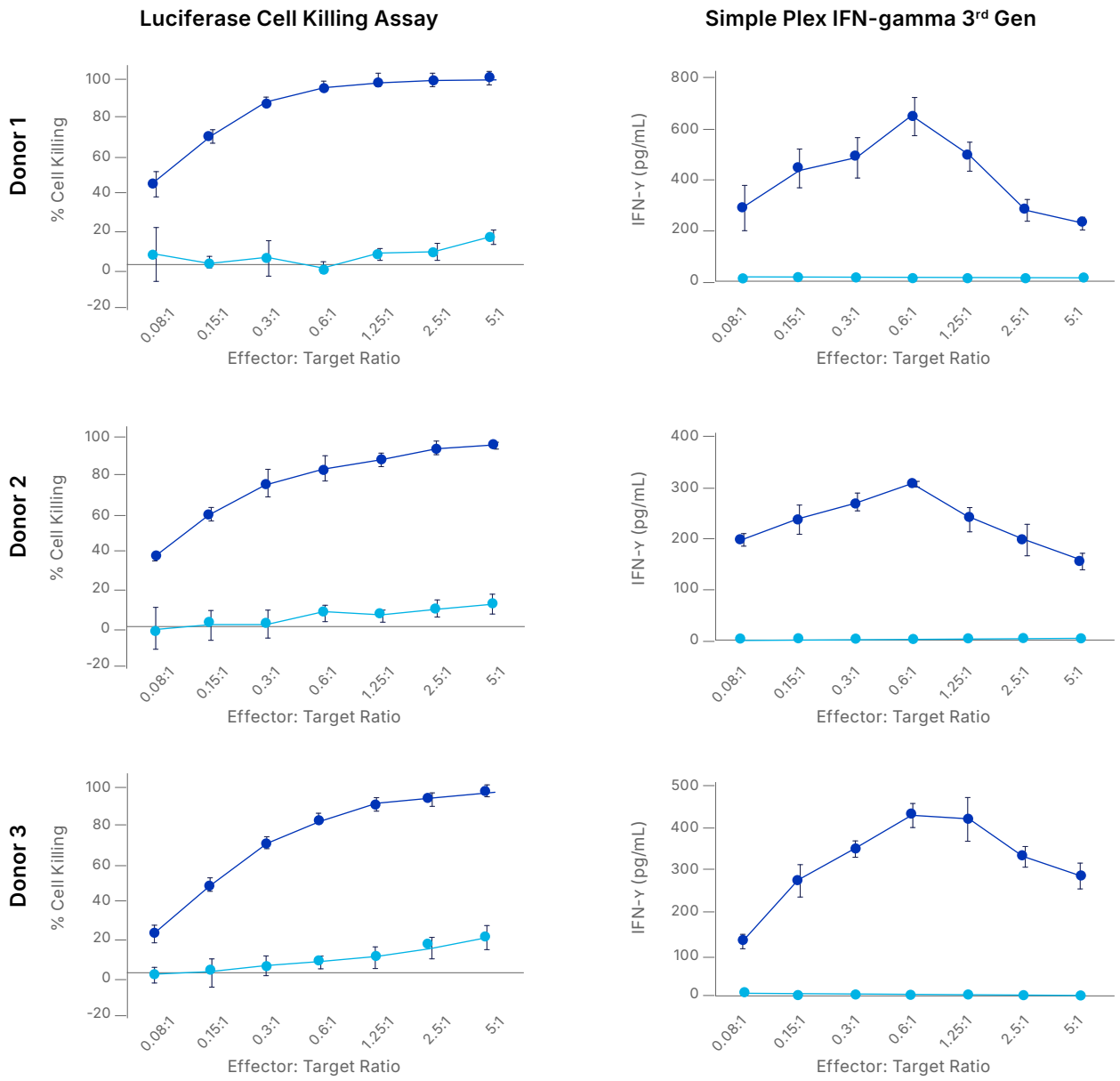

Figure 4. Cell lysis (measured by luciferase assay) and IFN-γ release (measured by Simple Plex assays) were observed in Nalm-6 co-cultures but not in K562 co-cultures. Anti-CD19 CAR-T cells from three donors were co-cultured with Nalm-6 cells (dark blue) or K562 cells (light blue) at varying E:T ratios. (Error bars indicate standard deviation. n = 3 replicate measurements for both methods)

Expanding the Cytokine Panel for Deeper Potency Insights

Beyond IFN-γ, other cytokines such as Granzyme B, IL-2, and TNF-α provide additional information about immune cell activation and potency. Using Simple Plex assays, we analyzed cytokine secretion from lentivirus edited CAR-T cells following co-culture with Nalm-6 targets (see Figure 5). In all cases, edited cells demonstrated higher cytokine secretion than unedited controls, with reproducible results across multiple donors.

FIGURE // 05

Cytokine Secretion Profiles

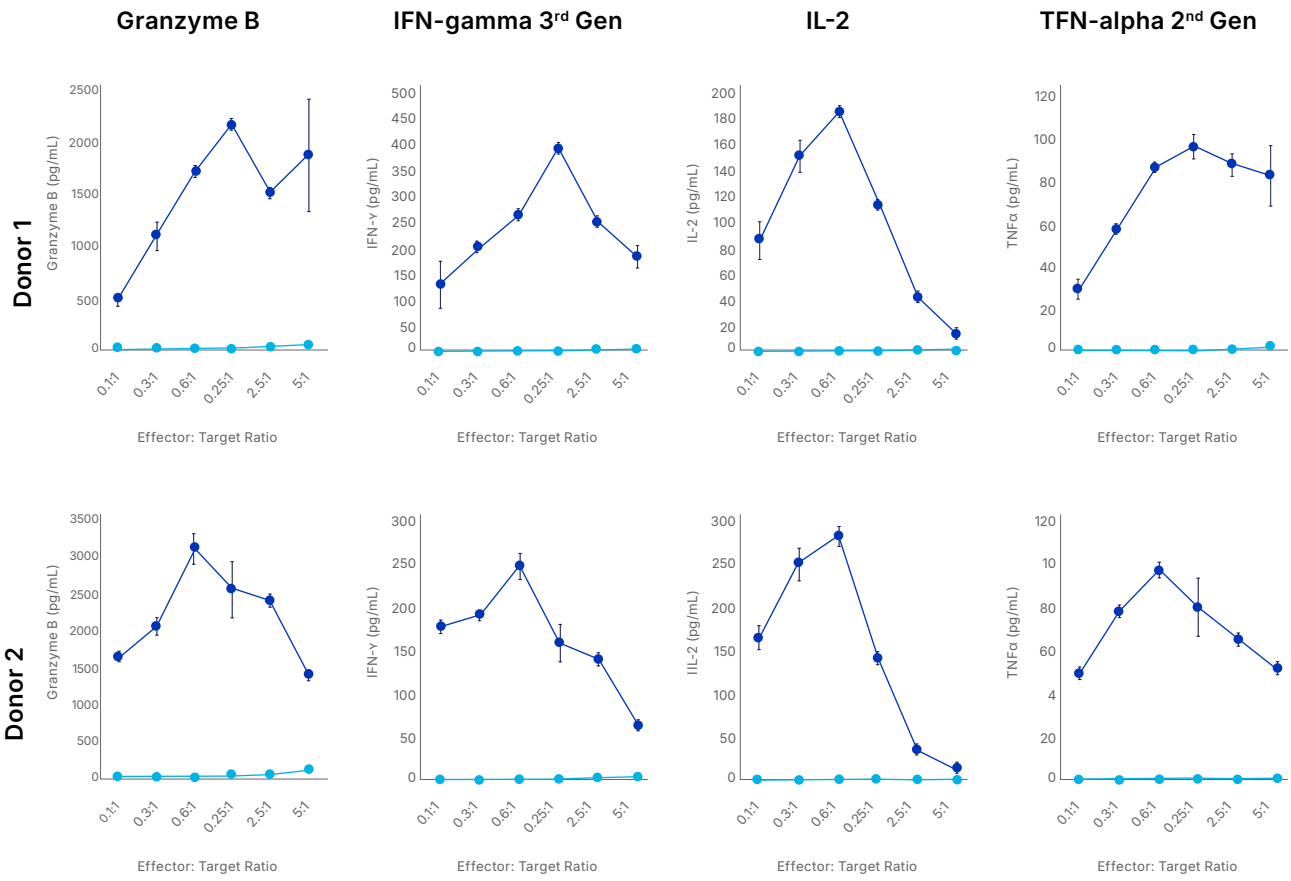

Figure 5. Cytokine secretion profiles from two CAR-modified donors (top and bottom rows) were measured using Simple Plex assays. T cell activation markers were elevated upon incubation of Nalm-6 cells with edited anti-CD19 CAR-T cells (dark blue) compared to unedited controls (light blue). Error bars indicate the range of 2 replicate measurements.

Method note: Lentivirus IL-7/IL-15

Conclusion

The Simple Plex Platform can deliver rapid, quantitative measurements of cytokine secretion from CAR-T cells, making it highly effective for potency testing in co-culture models. The precision, sensitivity, and consistency of the IFN-γ assay, along with its agreement with orthogonal assessments such as luciferase-based cytotoxicity assays, reinforce its value as a primary potency release method. Additionally, the ability to multiplex cytokine detection provides a more nuanced view of immune activation, supporting both mechanistic insights and product comparability.

Finally, the Simple Plex Platform is a scalable, automation-ready solution that supports evolving regulatory expectations and the increasing demands of clinical-grade cell therapy manufacturing. With fully automated workflows, validated performance, and the flexibility to assess multiple analytes, Simple Plex Platform supports high-throughput potency testing for clinical and manufacturing applications.

Learn More

Scan the QR Code or Contact:
bio-techne.com/resources/instrument-applications/simple-plex-cell-therapy

References

1. Guidance for Industry: Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products, FDA, Jan 2024
2. Draft Guidance for Industry: Potency Assurance for Cellular and Gene Therapy Products, FDA, Dec 2023