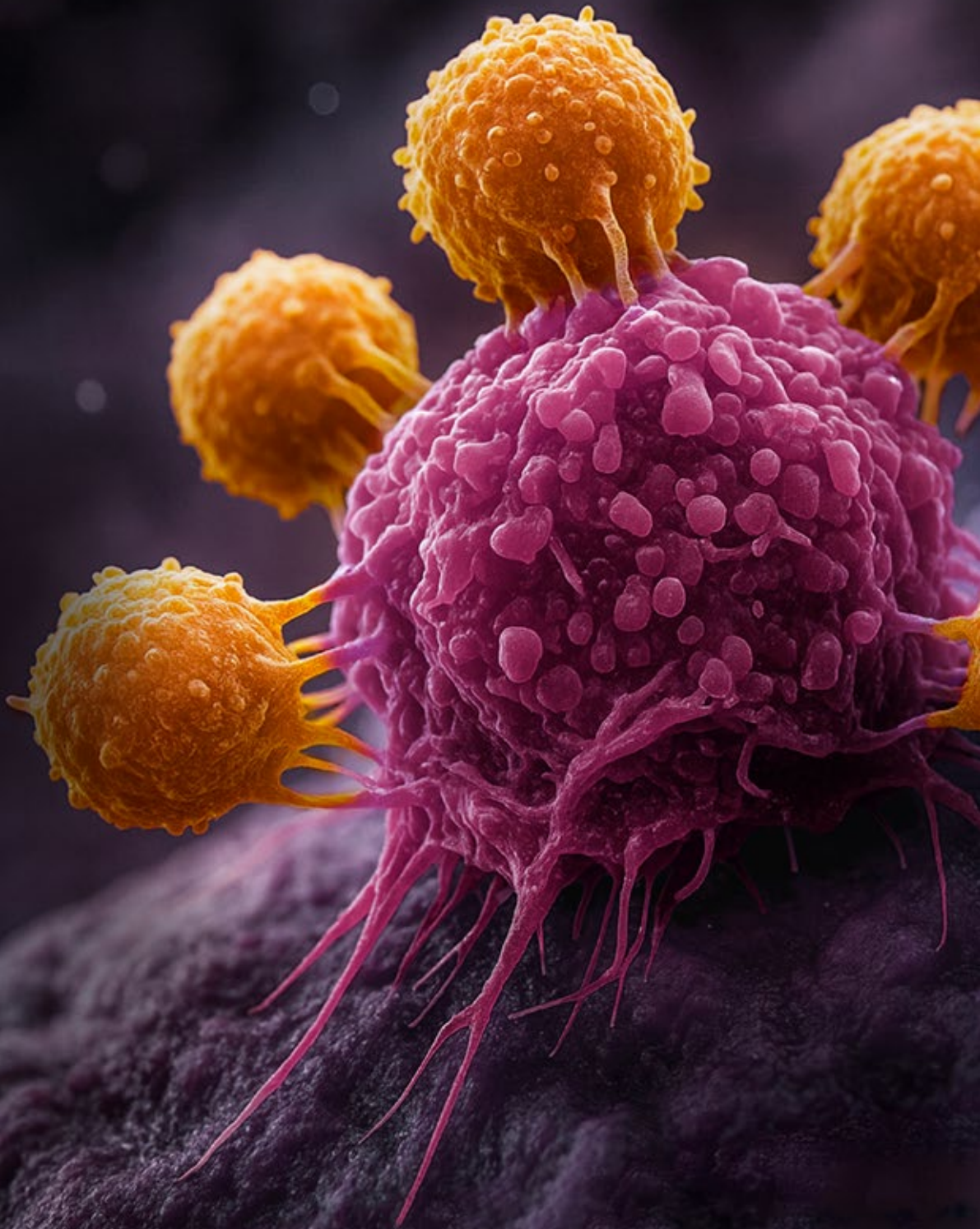


Cell & Gene Therapy

Powerful Tools for T Cell Therapy
Development



GLOMAX® DETECTION SYSTEMS

A Versatile, Reliable, and Intuitive Lab Companion to Support Your Research

GloMax® Discover is an advanced multimode plate reader designed to provide optimal performance for Promega reagents with high-performance luminescence, fluorescence, UV-visible absorbance, BRET and FRET, two-color filtered luminescence, and kinetic measurement capabilities. GloMax® Discover can be used as a standalone plate reading instrument or integrated into high-throughput automated workflows. Results are easy to interpret using integrated data analysis software.

One Instrument. Numerous Applications in Cell & Gene Therapy.

- Cell viability, proliferation and cytotoxicity measurement
- Cell metabolism profiling
- Cytokine detection with Lumit® Immunoassays
- Target cell killing analysis using HiBiT technology
- Potency assessment with luminescent T cell activation assays

GloMax

SYSTEMS

A high-performance, easy-to-use multimode plate reader for luminescence, fluorescence, absorbance, BRET, and FRET applications



For more information visit www.promega.com/gloMAX-comparison

Table of Contents

1	Cell & Gene Therapy Solutions	4
1.1	From discovery to drug release – solutions for every step	4
2	Cell Health & Metabolism Assays	6
2.1	Are my cells alive and actively proliferating?	7
2.2	Are my cells showing the expected metabolic activity?	10
3	Cell Potency Analysis	14
3.1	Do my cells produce the expected cytokines?	15
3.2	How potent are my cells in target cell killing?	18
3.3	How can I identify and validate functional TCRs and CARs with confidence?	24
4	Cell Tracking & Interaction Analysis in vivo	30
4.1	How can I track cell populations over time in live animals?	30
5	STR Genotyping in Cell Therapy Quality Control	32
5.1	How to use STR genotyping for quality control in cell therapy?	32
6	Ordering Information	38
7	Contacts	44

1. Cell & Gene Therapy Solutions



1.1 From Discovery to Drug Release – Solutions for Every Step

Cell and gene therapies are reshaping the way we treat cancer, genetic diseases, and other serious conditions. But developing these therapies is complex, requiring robust tools to assess critical quality attributes, meet evolving regulatory expectations, and ensure safe and effective products.

Wherever you are in your development process, Promega assays and technologies deliver the clarity and confidence you need to move forward, together with responsive support and tailored solutions.

Why Partner with Promega for Cell & Gene Therapy Development

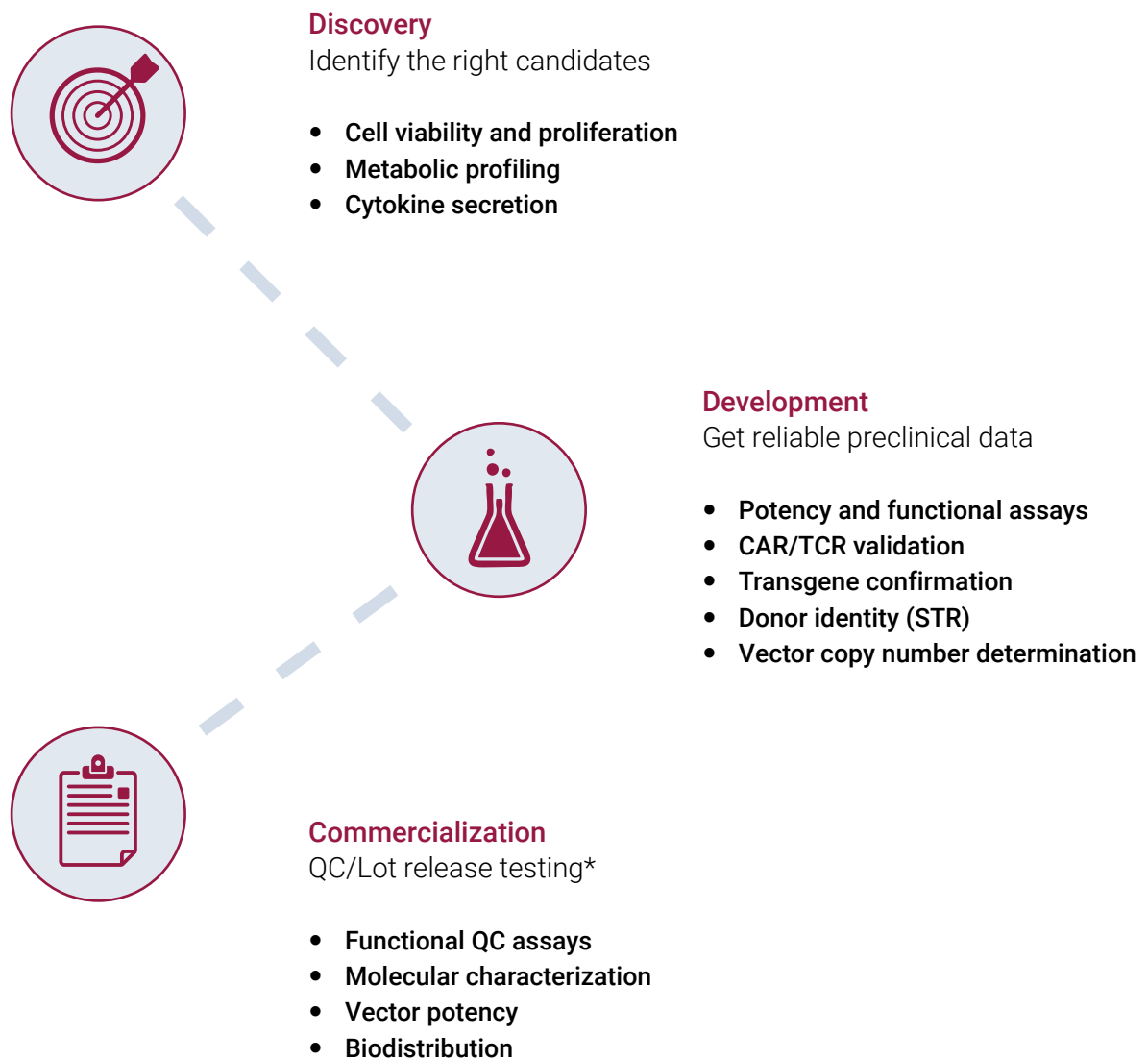
- **Validated assay performances** for reproducible results
- **Streamlined workflows** using homogeneous, add-mix-measure formats
- **Broad assay compatibilities** with various sample types and cell systems
- **Regulatory alignment**, with assays that support ICH compliance and GMP-adjacent use
- **Technical support** for assay implementation, troubleshooting, and optimization
- **Custom development services** to tailor assays, reagents, and workflows to your needs



Navigating Through the Development of Cell & Gene Therapies

Advancing a therapy from early discovery to final product release requires a clear view of critical milestones. Each phase – Discovery, Development, and Commercialization – comes with its own challenges and data requirements.

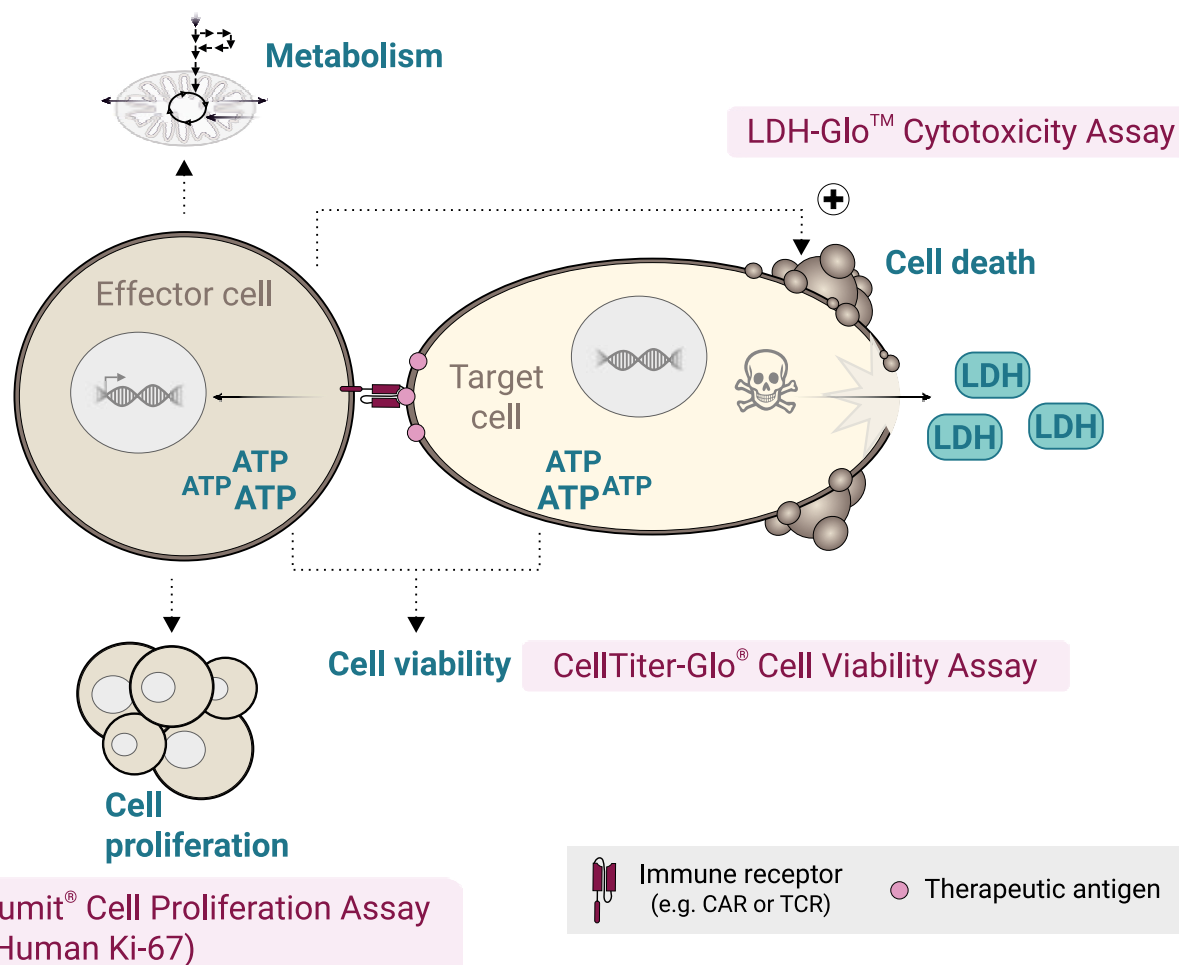
This roadmap outlines how Promega technologies can support key steps along the way, from identifying suitable candidates and validating function to ensuring product consistency and compliance.



* Assay suitability for QC/lot release testing depends on product validation and intended use. Please contact us for details.

Cell health and metabolism directly influence efficacy and safety in cell therapies. Our diverse portfolio of sensitive add-mix-read assays can help you answer questions about the **viability, proliferation and metabolism** of your cells.

Metabolism-Glo™ Assays



Key Questions

2.1 Are my cells alive and actively proliferating?	7
2.2 Are my cells showing the expected metabolic activity?	10



2.1 Are my cells alive and actively proliferating?

Monitoring cell health is crucial to ensure the efficacy, safety, and the overall therapeutic potential of cell and gene therapies. Luminescence-based assays provide sensitive, reproducible measurement of cell viability, proliferation, and cytotoxicity. These homogeneous assays are easy to use, scalable, and compatible with a wide range of cell types.

CellTiter-Glo® Cell Viability Assay

The **CellTiter-Glo® Assay** measures intracellular ATP, a universal marker of metabolically active (viable) cells. The lytic assay uses a luciferase reaction that converts ATP into a luminescent signal. The light output is directly proportional to the number of viable cells. **The CellTiter-Glo® 3D Assay** is specifically optimized to measure cell viability in various 3D culture formats, providing consistent and robust results in these complex and biologically relevant systems.

Lumit® Cell Proliferation Assay (Human Ki-67)

Ki-67 is a nuclear protein expressed during all active phases of the cell cycle (G1, S, G2, M), but absent in resting (G0) cells, making it a gold-standard marker for proliferation. The **Lumit® Cell Proliferation Assay** enables rapid, sensitive quantification of endogenous Ki-67 levels using a no-wash, luminescent format. The assay uses two Ki-67-specific antibodies, each conjugated to a complementary subunit of the NanoBiT® Luciferase. Upon binding to the antigen, the subunits reconstitute an active luciferase enzyme, generating a luminescent signal that is directly proportional to the amount of Ki-67 in the sample. This approach allows early detection of proliferation activity. For more information about the Lumit® technology see page 15.

LDH-Glo™ Cytotoxicity Assay

LDH (lactate dehydrogenase) is released into the culture medium upon loss of membrane integrity, which occurs during cell death. The **LDH-Glo™ Assay** converts LDH activity into a stable luminescent signal through a coupled enzymatic reaction. Useful for quantifying effector-target cell killing (e.g., ADCC, CAR-T cytotoxicity) without labeling cells or modifying culture conditions.

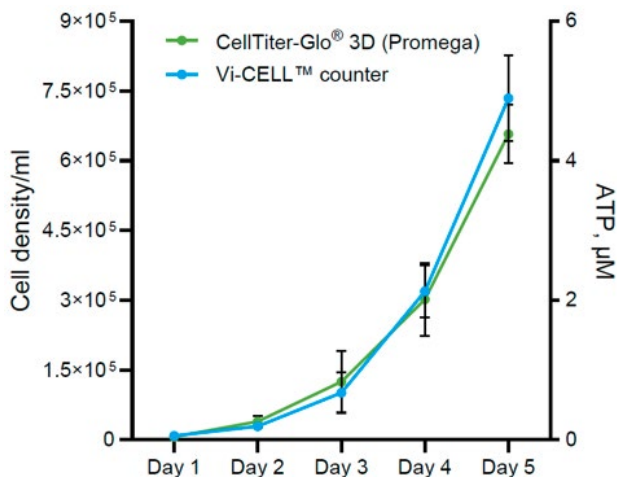
2. Cell Health & Metabolism Assays



2.1 Are my cells alive and actively proliferating?

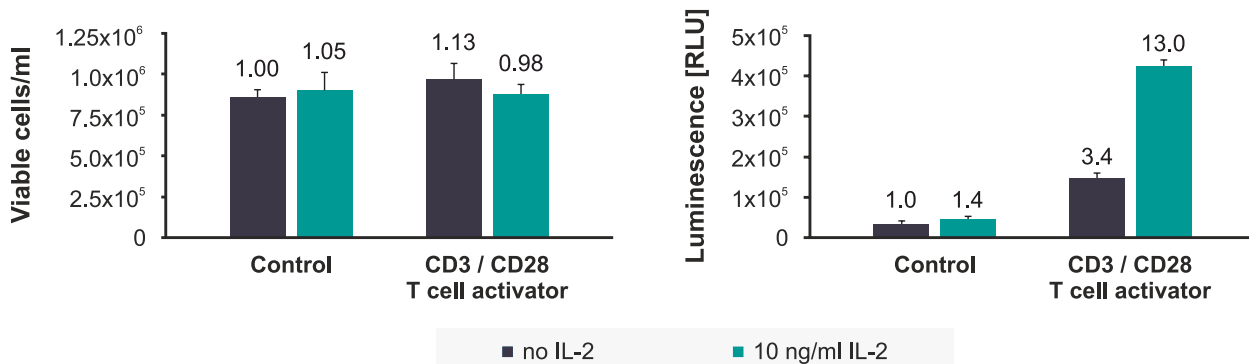
Application Examples:

Monitoring T Cell Expansion Using CellTiter-Glo® 3D Assay



Human adipose-derived stem cells (hADSCs) were expanded over 5 days in a BioBLU® 0.3c Single-Use Bioreactor. Cell viability was measured using the **CellTiter-Glo® 3D Assay** (green) and compared to counts obtained from a Vi-CELL™ automated cell counter (blue). The luminescent ATP signal strongly correlates with cell density, demonstrating the assay's suitability for bioprocess monitoring. Data represent the average of six bioreactors (n=6).

Detecting Early T Cell Activation with Lumit® Cell Proliferation Assay



Human CD8⁺ T cells (StemCell Tech) plated at 80,000 cells per well were treated with CD3/CD28 T cell activator with (green) and without (dark gray) 10 ng/ml IL-2 for 48 hours. Upregulation of Ki-67 is observed with the **Lumit® Cell Proliferation Assay** before T cell proliferation (which begins >72 hours after activation), demonstrating use of this assay as an early indicator of proliferation.



References

Dahlke, J. et al. (2021) Efficient genetic safety switches for future application of iPSC-derived cell transplants. *J. Pers. Med.* 11, 565.

Wu, Y. et al. (2022) A new immunotherapy strategy targeted CD30 in peripheral T-cell lymphomas: CAR-modified T-cell therapy based on CD30 mAb. *Cancer Gene Ther.* 29, 167–177.

Li, H.K. et al. (2023) A novel allogeneic Rituximab-conjugated gamma delta T cell therapy for the treatment of relapsed/refractory B-cell lymphoma. *Cancers* 15, 4844.

Product Box

CellTiter-Glo® Luminescent Cell Viability Assay	Cat. # G7572
CellTiter-Glo® 2.0 Cell Viability Assay	Cat. # G9242
CellTiter-Glo® 3D Cell Viability Assay	Cat. # G9683
Lumit® Cell Proliferation Assay (Human Ki-67)	Cat. # GC1000
LDH-Glo™ Cytotoxicity Assay	Cat. # J2381



A more detailed product list can be found on pages 34 – 37.

Looking for the right assay for your experiment?



Download this brochure to discover our full portfolio of cell-based and biochemical assays.

www.promega.com/CellbasedAssays

2. Cell Health & Metabolism Assays



2.2 Are my cells showing the expected metabolic activity?

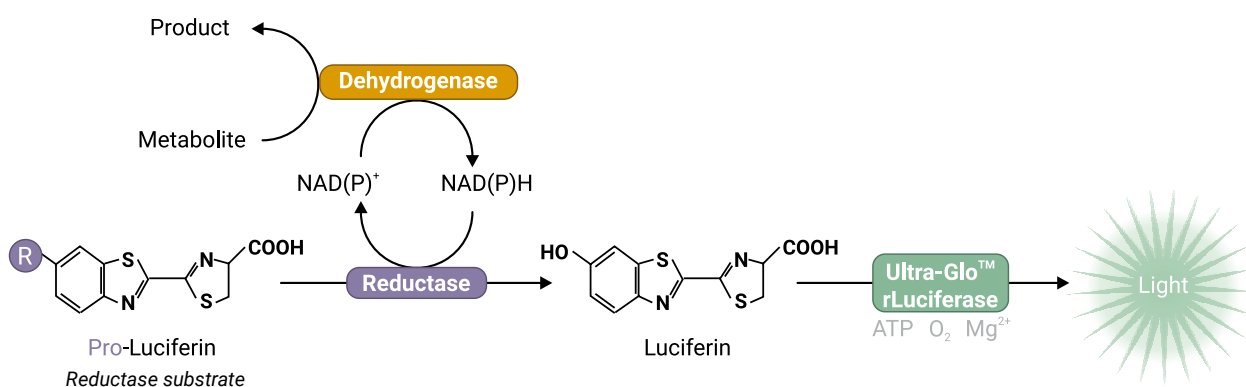
Cellular metabolism plays a pivotal role in the development and manufacturing of cell therapies. It influences key processes such as cell proliferation, differentiation, and effector function. By understanding and optimizing metabolic pathways, researchers can improve cell yield, monitor functional states, and identify phenotypic shifts relevant for quality control and clinical performance.

Bioluminescent Metabolism Assays – Fast, Sensitive, Selective

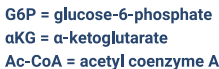
Cellular metabolism assays target critical areas such as glucose metabolism, mitochondrial function, amino acid and lipid metabolism, and redox balance, providing comprehensive insights into cellular metabolic activity.

Most assays rely on oxidation of a specific metabolite, coupled with NAD(P)^+ reduction and subsequent light production through a luciferase reaction. The light signal is directly proportional to the metabolite concentration. The assays are compatible with various sample types, requiring only simple dilution and/or acid treatment followed by neutralization. The simple add-mix-measure protocols enable rapid analysis of large sample sets.

Assay Principle



To detect most metabolites, oxidation is coupled via a metabolite-specific dehydrogenase with a concomitant reduction of NAD(P)^+ to NAD(P)H . The light produced is proportional to the amount of metabolite in the sample.



- **Add-mix-measure format:** No extraction or organic extraction
- **Compatible with different sample types** including organoids, tissues and plasma
- **Multiplexing options:** Measure several metabolites from one sample
- **High-throughput compatible**

2. Cell Health & Metabolism Assays

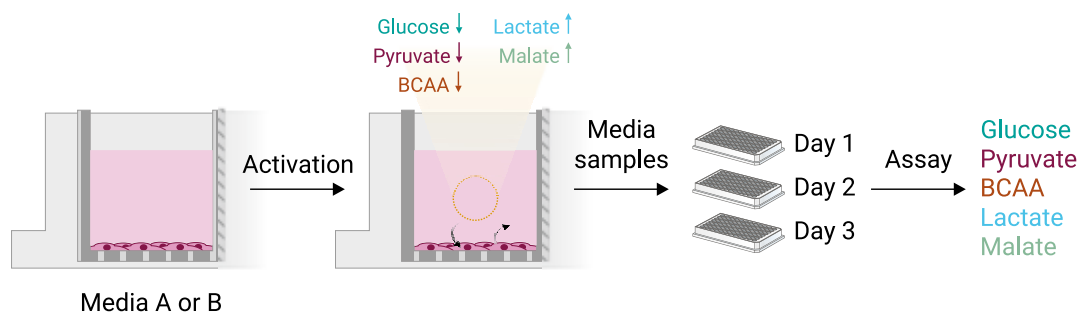
2.2 Are my cells showing the expected metabolic activity?

Application Examples:

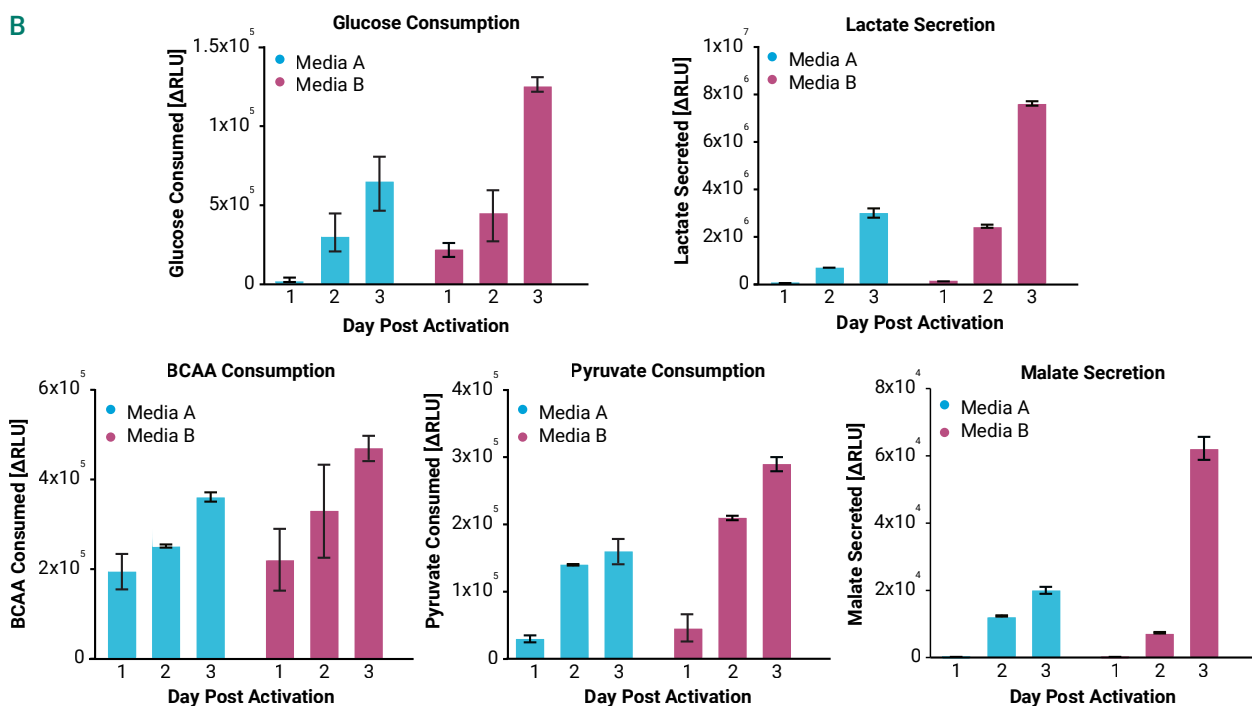
Profiling T Cell Metabolism Under Different Culture Conditions

T cells with a stem cell memory phenotype (Tscm) are known for their prolonged *in vivo* presence and function, which is a key characteristic for immunotherapies like CAR-T. To support Tscm enrichment, culture conditions must be carefully optimized and monitored. This example illustrates how metabolic measurements can serve as early predictors of T-cell phenotypic profiles.

A



B



(A) T cells were activated and cultured in two media formulations (Media A and Media B). Media samples were collected on days 1, 2, and 3 post-activation and analyzed with the Glucose-, Lactate-, BCAA-, Pyruvate-, and Malate-Glo™ Assays. (B) Results show differences in glucose and pyruvate consumption, as well as lactate, malate, and BCAA secretion, reflecting media-dependent shifts in metabolic profiles.

References

Cappabianca, D. et al. (2024) Metabolic priming of GD2 TRAC-CAR T cells during manufacturing promotes memory phenotypes while enhancing persistence. *Mol. Ther. Methods Clin. Dev.* 32(2), 101249.

Sylvester, K. et al. (2025) Defined metabolic states shape T cell fate and function across culture conditions. *Front. Immunol.* 16,1703095.

Product Box

Glucose Uptake-Glo™ Assay	Cat. # J1341
Glucose-Glo™ Assay	Cat. # J6021
Lactate-Glo™ Assay	Cat. # J5021
Glycogen-Glo™ Assay	Cat. # J5051
Pyruvate-Glo™ Assay	Cat. # J4051
Malate-Glo™ Assay	Cat. # JE9100
Glutamine/Glutamate-Glo™ Assay	Cat. # J8021
BCAA-Glo™ Assay	Cat. # JE9300
Triglyceride-Glo™ Assay	Cat. # J3160
Cholesterol/Cholesterol Ester-Glo™ Assay	Cat. # J3190
BHB-Glo™ Assay	Cat. # JE9500
ROS-Glo™ H ₂ O ₂ Assay	Cat. # G8820
NADP/NADPH-Glo™ Assay	Cat. # G9081
NAD/NADH-Glo™ Assay	Cat. # G9071



A more detailed product list can be found on pages 34 – 37.

Looking for a specific metabolite?



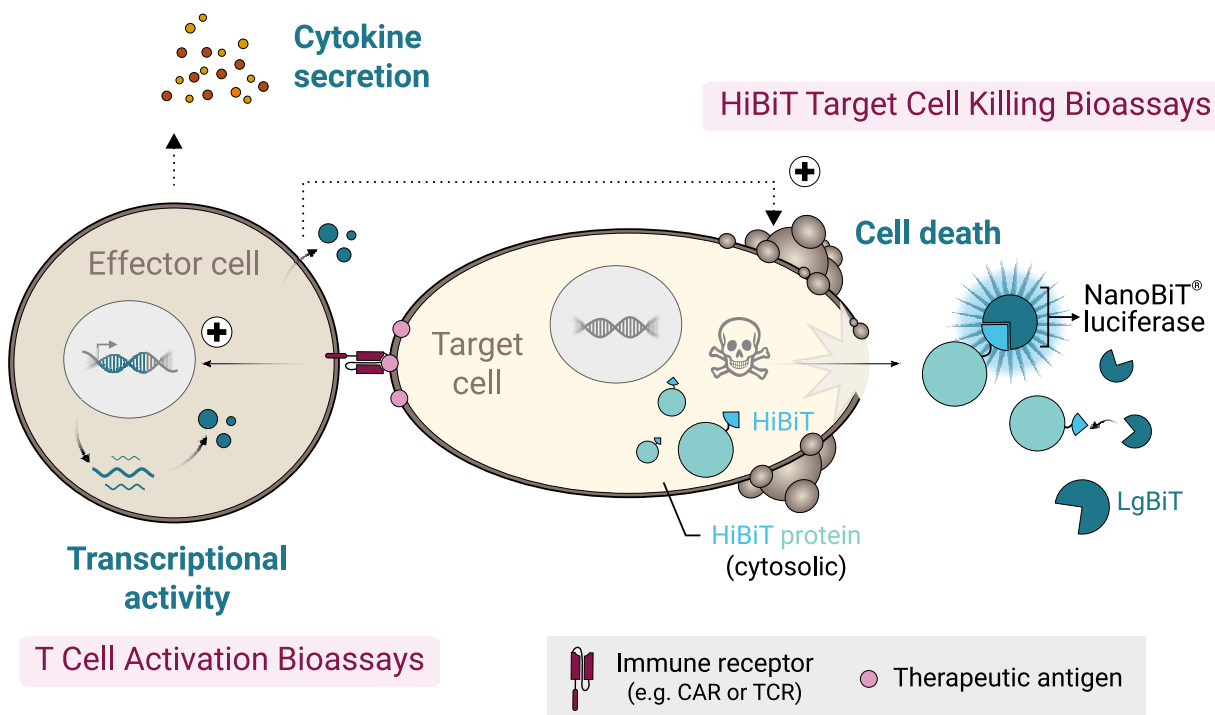
With the **Metabolite-Glo™ Detection System (Cat.# J9030)** you can design a customized bioluminescent metabolism assay tailored to your target of interest. Discover more about this assay and explore our complete portfolio of bioluminescent assays at

www.promega.com/CellularMetabolismAssays

3. Tools for Potency & Functional Analysis

Potency and functional assays are fundamental to the development of cell therapies, as they provide critical insights into the therapeutic potential and consistency of the cell products. Potency assays measure the biological activity of the cells, ensuring they can achieve the desired therapeutic effect, while functional assays assess the specific capabilities of the cells, such as their ability to differentiate, secrete factors, or target diseased cells. Together, these assays are essential **for validating the efficacy, safety, and reliability of cell and gene therapies**.

Lumit[®] Cytokine Immunoassays



Key Questions

3.1 Do my cells produce the expected cytokines?	15
3.2 How potent are my cells in target cell killing?	18
3.3 How can I identify and validate functional TCRs and CARs with confidence?	24

3.1 Do my cells produce the expected cytokines?

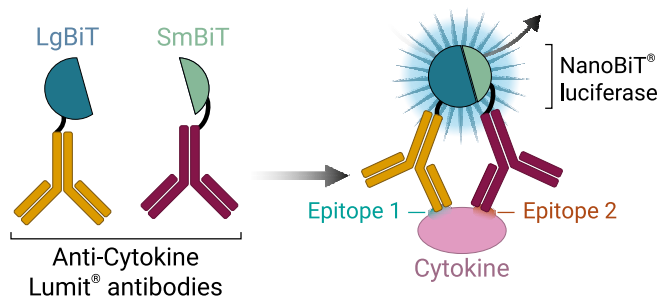
Cytokine secretion is a key functional hallmark of activated T cells and other immune effector cells used in cell and gene therapy. Measuring cytokines like IL-2 and IFN- γ provides critical insights into cell potency, immune engagement, and therapeutic efficacy. Whether you're optimizing T cell engagers, validating CAR-T cell function, or performing release testing – cytokine quantification is essential.

Lumit® Cytokine Immunoassays – Fast, Sensitive, and Wash-Free

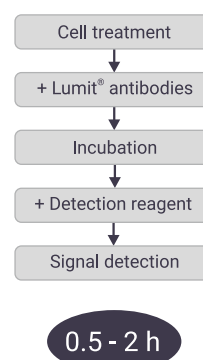
The Lumit® technology is a simple and fast alternative to run homogeneous immunoassays in multi-well plate formats. The underlying principle is based on the NanoLuc® Binary Technology (NanoBiT®) (see page 17). In **Lumit® Immunoassays**, two antibodies are chemically labeled with the small and large subunits of NanoLuc® Luciferase, SmBiT and LgBiT, respectively. Direct or indirect binding to the analyte yields spatial proximity of the labeled antibodies and enables SmBiT and LgBiT to reconstitute the NanoBiT® Luciferase. In the presence of its substrate furimazine, a bright luminescence can be detected that is directly proportional to the amount of analyte present in the sample.

The Lumit® workflow enables fast and sensitive detection without washing steps. Simply add labeled antibodies and detection reagent to your cell culture supernatant, and measure luminescence to quantify your target analyte in just 70 minutes or less.

Assay Principle



No-Wash Workflow



Lumit® Immunoassays use specific primary antibodies labeled with the NanoBiT® Luciferase subunits SmBiT and LgBiT. When the analyte is present, the subunits join to form an active luciferase enzyme, producing a bright luminescent signal. Unlike ELISA, which requires immobilization and wash steps, Lumit® assays self-assemble and allow direct detection in the assay medium.

3. Tools for Potency & Functional Analysis

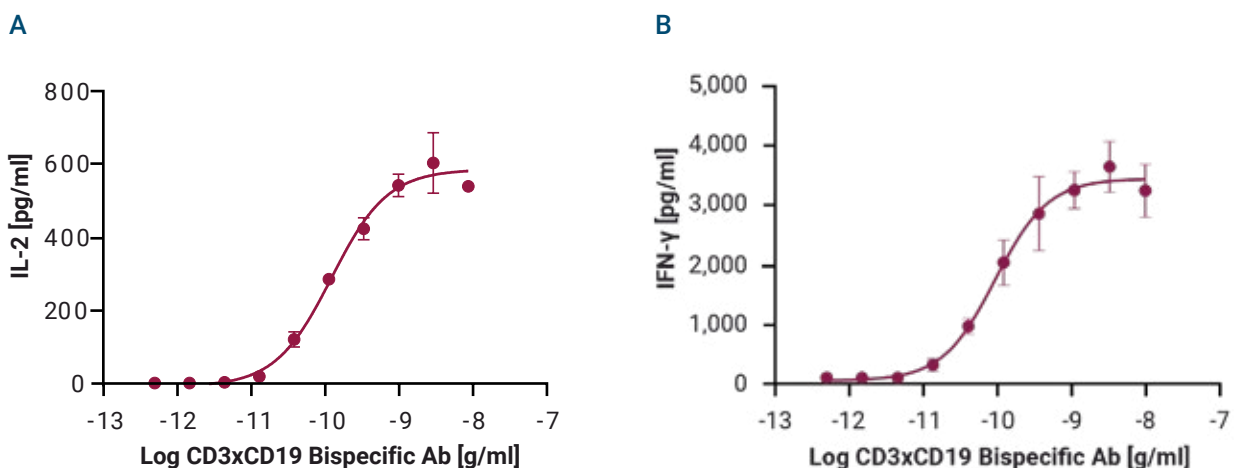
3.1 Do my cells produce the expected cytokines?

Key Benefits of Lumit® Immunoassays for Cytokines

- **Simple add-and-read** protocol with no washing steps or immobilization
- **Fast results** in 70 minutes or less
- **Direct analyte measurement** in cell culture plates
- **Sensitive luminescence detection** with a **wide dynamic range**
- Detection on a conventional **luminometer**
- **High-throughput compatible** (96-well and 384-well plates)

Application Examples:

Lumit® Immunoassays measure IL-2 and IFN-γ production from activated CD8⁺ T cells



Purified CD8⁺ T cells (effector cells) were combined with Raji B cells (target cells) and a serial dilution of Blincyto® (CD3xCD19 bispecific T cell engager). IL-2 (A) and IFN-γ (B) levels were measured with the corresponding Lumit® Cytokine Immunoassays to detect cytokine release from the effector cells.

Product Box

Lumit® IL-2 (Human) Immunoassay

Cat. # W6020

Lumit® IFN-γ (Human) Immunoassay

Cat. # W6040

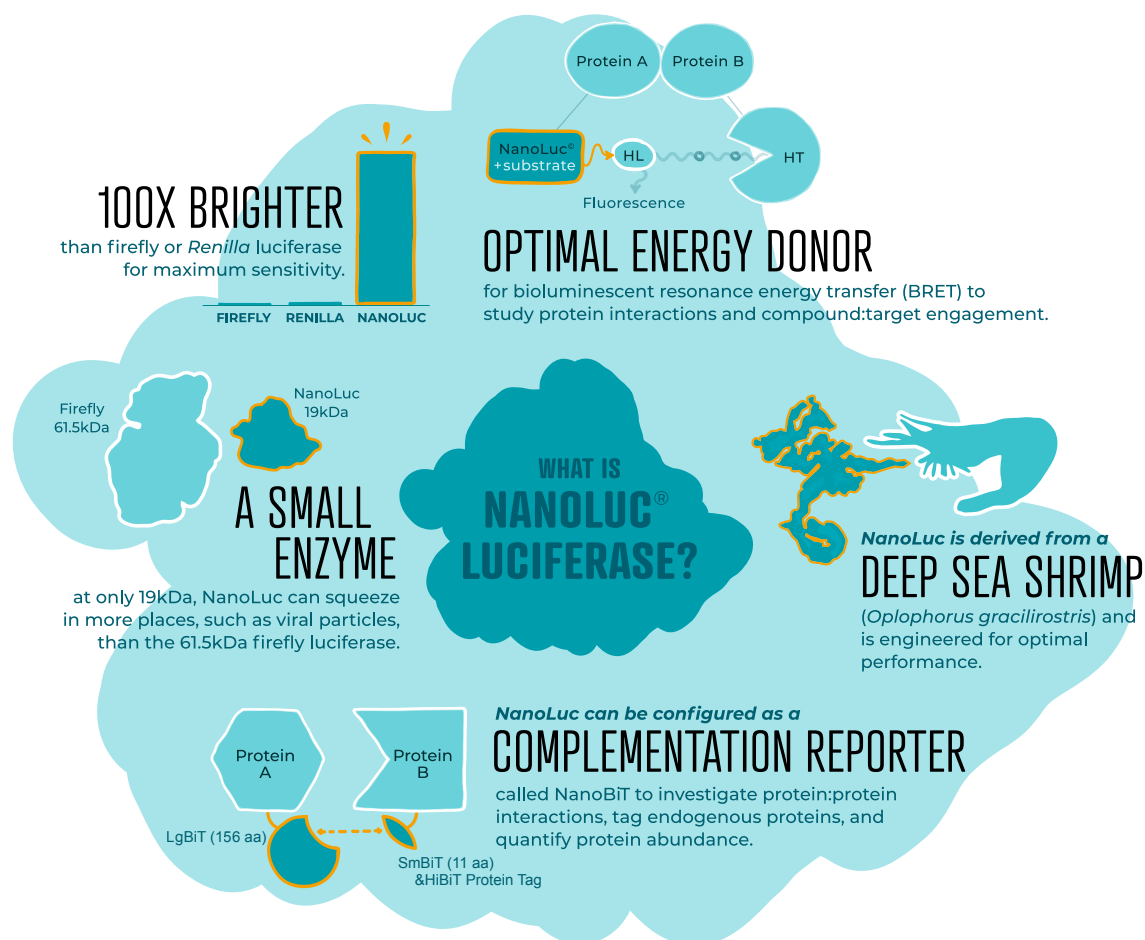


A more detailed product list can be found on pages 34 – 37.

Technology Spotlight

NanoLuc® Luciferase: One Enzyme, Endless Capabilities

Many Promega functional assays are powered by **NanoLuc® Luciferase**, a small engineered luciferase that delivers exceptionally bright and stable luminescent signals.



NanoLuc® Technology Enables a Wide Range of Applications:

- Protein Kinetic Analysis
- Real-time Protein Interaction Assays
- Cellular Target Engagement Assays
- Genetic Reporter Studies
- Apoptosis Detection
- Lumit® Immunoassay Detection
- HiBiT Target Cell Killing Bioassays

Image NanoLuc® with GloMax® Galaxy Bioluminescence Reader:



www.promega.com/GloMaxGalaxyDE

GloMax
G A L A X Y

3. Tools for Potency & Functional Analysis

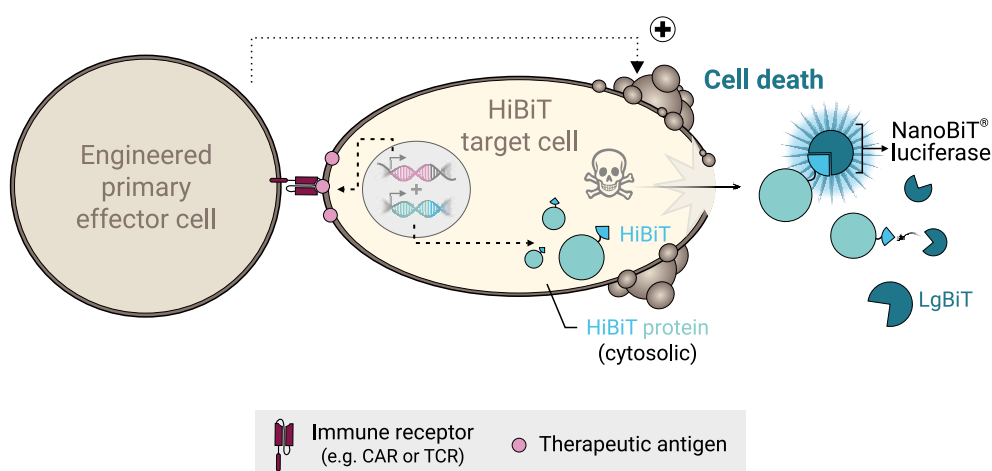
3.2 How potent are my cells in target cell killing?

The therapeutic efficacy of engineered T-cell products such as CAR-T and TCR-T cells depends on their ability to recognize and eliminate target cells with precision. Accurate assessment of this cytotoxic function is critical for developing safe and consistent cell therapies. However, conventional cytotoxicity assays often struggle to differentiate between target and effector cell signals, particularly in complex co-culture systems. The HiBiT Target Cell Killing (TCK) Bioassay overcomes these limitations by directly and specifically quantifying target cell death. This enables a highly precise and reproducible measurement of CAR-T or TCR-T cell-mediated killing.

HiBiT Target Cell Killing (TCK) Bioassays

The **HiBiT Target Cell Killing (TCK) Bioassays** provide a sensitive, mechanism-based method for quantifying the cytotoxic activity of effector cells in cell therapy research. The assay is based on engineered target cell lines that stably express a HiBiT fusion protein. HiBiT is an 11-amino acid peptide tag with strong affinity for the complementary LgBiT subunit of NanoBiT® Luciferase. When effector cells, such as CAR-T or TCR-T cells, which are supplied by the user, lyse the HiBiT-expressing target cells, the intracellular HiBiT peptide is released and binds to LgBiT, reconstituting an active luciferase enzyme. The reconstituted NanoBiT® Luciferase emits a stable and bright luminescent signal after reagent addition. The signal correlates with the number of dead cells, enabling a precise and reproducible measure of effector-cell-mediated-cytotoxicity in co-culture systems.

Assay Principle



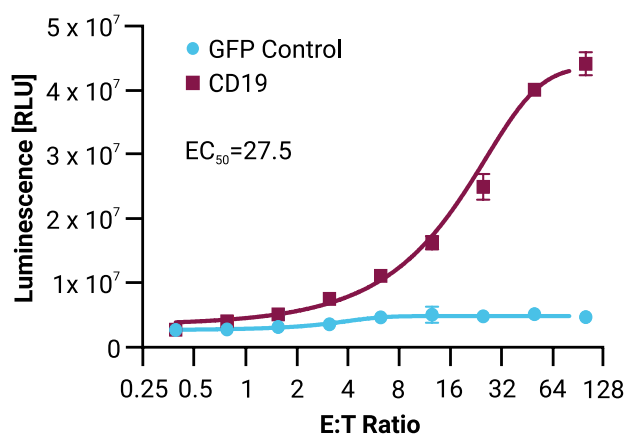
Effector cells (e.g., CAR-T or TCR-T, provided by the customer) are incubated with target cells expressing a HiBiT fusion protein. Upon killing of the target cell, the HiBiT fusion protein is released and binds extracellular LgBiT to create a functional NanoBiT® Luciferase enzyme. Luminescence is measured using a luciferase substrate and a luminometer.

Key Benefits of the HiBiT TCK Bioassay Platform

- **Mechanism-of-action (MOA)-based functional readout:** directly quantifies CAR-T and TCR-T cytotoxicity
- **High specificity in co-cultures:** measures target cell death without interference from effector cells
- **Quantitative, reproducible results:** For ICH-compliant potency testing
- **Flexible assay design:** Use ready-to-use HiBiT Target Cells or generate custom cells, e.g., via ViaSript™
- **GMP-adjacent compatibility:** Supports in-process control and lot release testing

Application Examples:

CAR-T Specificity and Dose Response Validation with HiBiT



T cells transduced with CAR-19 or a GFP control lentivirus were combined with HiBiT Target Cells (Ramos) at different E:T ratios and incubated for 24 hours. CAR-19 T cells induced robust, E:T ratio-dependent target cell killing, while GFP control T cells showed no detectable cytotoxicity, demonstrating the specificity of the assay.

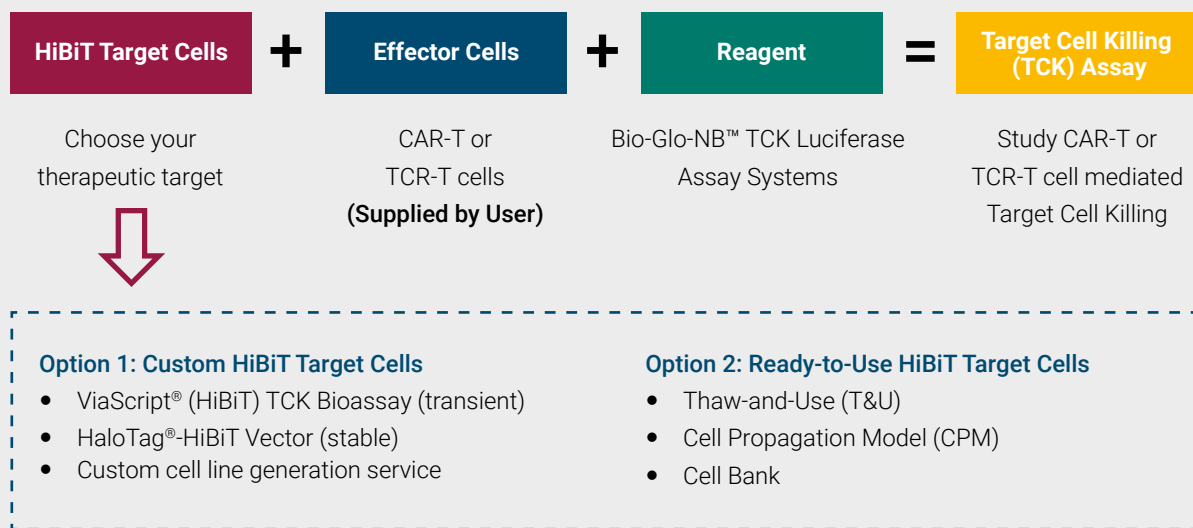
3. Tools for Potency & Functional Analysis

3.2 How potent are my cells in target cell killing?

HiBiT Target Cells

HiBiT Target Cells are provided as stable, prequalified ready-to-use cell lines, available either as a **cryopreserved Thaw-and-Use (T&U) format** or as a **Cell-Propagation Model (CPM)** (see pp. 22–23). Alternatively, **custom target cells** can be generated by stably or transiently expressing HaloTag®-HiBiT in the cell line of interest.

How to Build Your HiBiT Target Cell Killing (TCK) Bioassay



Custom HiBiT Target Cell Generation

Two flexible approaches enable the generation of custom HiBiT-expressing target cells:

Stable genomic integration of HiBiT (plasmid-based):

Generation of stably transfected cell lines using HaloTag®-HiBiT expression vectors (for ordering information see pages 34–37), providing durable HiBiT reporter expression for long-term studies, extended assays, or routine screening applications in your cell background of choice.

ViaScript® HiBiT TCK Bioassay (mRNA-based transient expression):

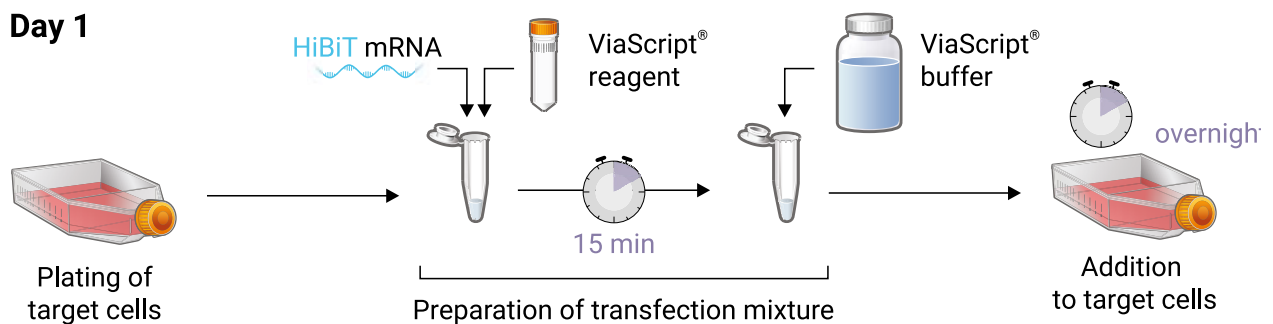
A low-toxicity, high-efficiency mRNA transfection method ideal for hard-to-transfect or patient-derived primary cell models. Enables rapid and transient HiBiT expression suitable for assay development and screening workflows (see next page).

ViaScript® HiBiT TCK Bioassay

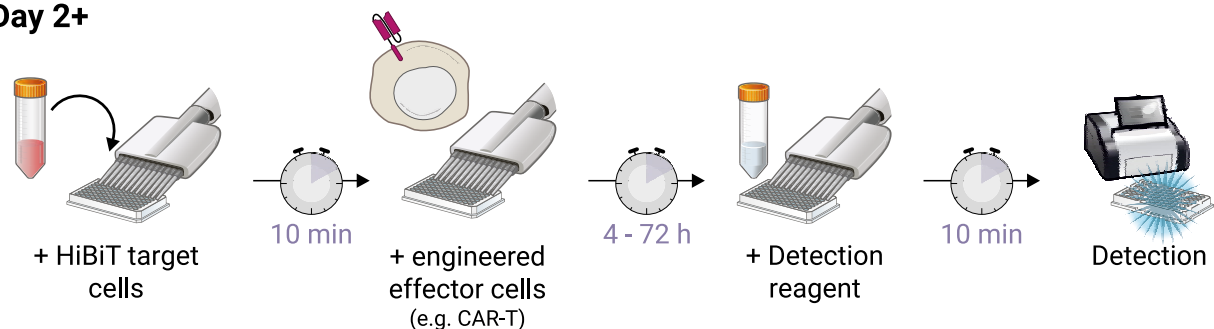
The **ViaScript® HiBiT TCK Bioassay** offers a flexible solution for generating custom HiBiT-expressing target cells. This low-toxicity, high-efficiency transfection system is optimized for delivering HiBiT-tagged constructs, enabling you to apply the sensitive HiBiT TCK Bioassay across a wider range of cell types. Ideal for labs developing personalized or hard-to-transfect models.

Workflow

Day 1



Day 2+



Day 1: Target cells are plated and transfected with HiBiT-tagged mRNA using the ViaScript® Reagent. The mRNA and ViaScript® Reagent are first combined to form a transfection mixture. This mixture is then combined with ViaScript® Buffer to create a complexation mixture, which is added to the cells to facilitate mRNA uptake and HiBiT expression.

Day 2+: The HiBiT-expressing target cells are transferred to assay plates. Effector cells (e.g., CAR-T, NK cells, or T cells) are added with or without biologics (e.g., monoclonal antibodies, BiTEs). Following co-culture for 4–72 hours, the detection reagent is added. After a 10-minute incubation, luminescence is measured using a plate reader to quantify cell killing based on the loss of HiBiT signal.

Product Box

ViaScript® (HiBiT) TCK Bioassay

Cat. # CS3628B19*



* This is an Early Access Material. Please enquire for more information.

3. Tools for Potency & Functional Analysis

3.2 How potent are my cells in target cell killing?

Prequalified Ready-to-Use HiBiT Target Cell Lines

Prequalified HiBiT-expressing target cells are available for most **popular immunotherapeutic targets** (e.g., CD19, CD20, BCMA, HER2, EGFR, and more) in three formats: **cryopreserved Thaw-and-Use Cells**, **Cell Propagation Models** and **Cell Banks**. Choose the format that best matches your workflow. For streamlined assay setup and consistent, reproducible results, Thaw-and-Use Cells eliminate the need for cell propagation – ideal for regulated environments.

List of Available Ready-to-Use HiBiT Target Cell Lines

Blood Cancer Target Cells

B Cell Leukemia



- Raji, Ramos
- Expressing CD19, CD20, CD22, CD38, CD70
- Knockout cell lines

Myeloid Leukemia



- U937 and K562
- Expressing: CD33, CLL-1

Multiple Myeloma



- H929
- Expressing: BCMA, CD38

T Cell Leukemia



- T2
- Expressing: CD5, CD7, CD30, CD52

Solid Tumor Target Cells

Ovarian Carcinoma



- OVAR3 and SKOV3
- Expressing: HER2, MSLN, ST4, MUC16
- MSLN-KD

Breast Adenocarcinoma



- SK-BR-3
- Expressing: HER2, EpCAM

Lung Carcinoma



- A549
- Expressing: EGFR

Melanoma



- A375
- Expressing: HER2, CD70, B7-H3

Pictures from biorender.com

Product Box

Ramos (HiBiT) TCK Bioassay	Cat. # JA1411
H929 (HiBiT) TCK Bioassay	Cat. # JA1511
Raji (HT-HiBiT) TCK Bioassay	Cat. # JA1211
Raji (HT-HiBiT) CD19-KO TCK Bioassay	Cat. # JA1251
Raji (HT-HiBiT) CD20-KO TCK Bioassay	Cat. # JA1261
Raji (HT-HiBiT) CD19/20-KO TCK Bioassay	Cat. # JA1271
Raji (LDH-HiBiT) TCK Bioassay	Cat. # JA1311
Raji (LDH-HiBiT) CD19-KO TCK Bioassay	Cat. # JA1221
Raji (LDH-HiBiT) CD20-KO TCK Bioassay	Cat. # JA1231
Raji (LDH-HiBiT) CD19/20-KO TCK Bioassay	Cat. # JA1241



All TCK Bioassays include the following components:

Fetal Bovine Serum, RPMI 1640 Medium, Bio-Glo-NB TCK Luciferase Assay Substrate, Bio-Glo-NB TCK Luciferase Assay Buffer, Bio-Glo-NB TCK LgBiT Protein.

A more detailed product list can be found on pages 34 – 37. Depending on the cell line, TCK Bioassays are available as Thaw-and-Use cells, Cell Propagation Models or Cell Banks. Additional assays may also be available through our Early Access Program and are therefore not listed in this brochure. For the complete, current list of available assays and formats, please contact Promega or your local sales representative.

Looking for a specific cell line?



Scan the QR code or visit our website to explore available **HiBiT Target Cells**.

www.promega.com/HiBiT-target-cell-killing

3. Tools for Potency & Functional Analysis

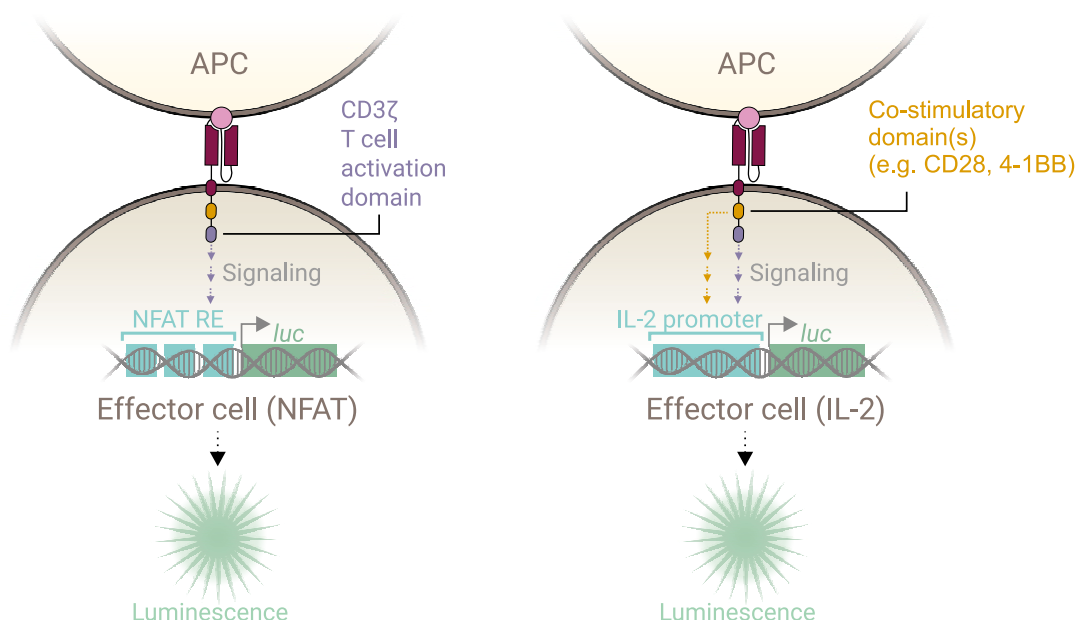
3.3 How Do I Screen and Validate New CARs or TCRs?

The development of CAR-T and TCR-T cells requires not only confirmation of receptor expression but also demonstration of functional activity. T Cell Activation Bioassays provide an early, sensitive and quantitative readout of receptor engagement and downstream signaling, enabling rapid screening, target validation and functional characterization of new CAR and TCR constructs. These assays complement HiBiT TCK Bioassays, which quantify downstream target cell killing as a functional potency readout. In addition to discovery applications, this mechanism-of-action-based activation readout can also be used to assess the functional potency of lentiviral CAR products.

T Cell Activation Bioassays

These assays use engineered Jurkat T cells expressing luciferase reporters driven by NFAT response elements or by the IL-2 promoter. Upon receptor engagement, intracellular signaling induces luciferase expression, and the resulting bioluminescent signal correlates with T cell activation and functional response. By introducing a CAR transgene into the reporter cells, for example via lentiviral transduction, these assays can be used to quantify CAR-mediated activation by measuring downstream signaling upon CAR:target engagement.

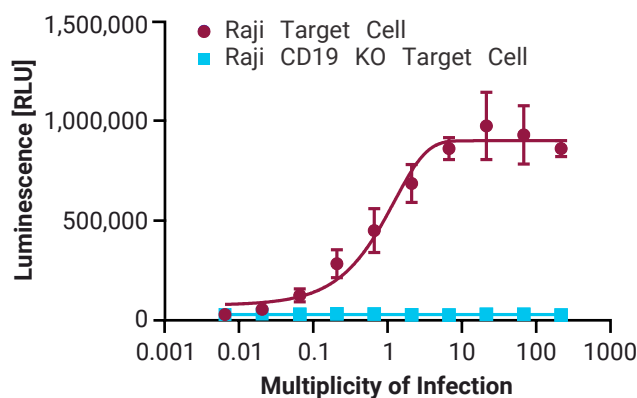
Assay Principle



The **T Cell Activation Bioassays (NFAT and IL-2)** each consist of a genetically engineered cell line. The NFAT reporter provides an early readout of TCR-mediated signaling, whereas the IL-2 reporter integrates TCR signaling and co-stimulatory signals, providing a readout of a later stage of T cell activation.

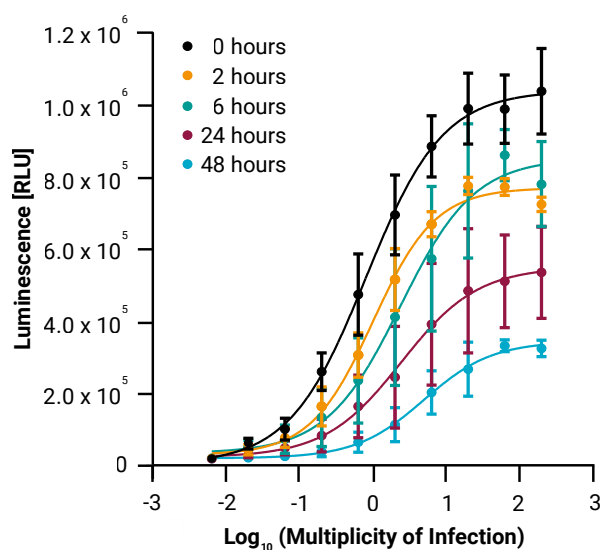
Application Examples:

CAR-T Cell Activation and Assay Specificity: Luciferase-based T Cell Activation Bioassays enable sensitive, MOA-aligned quantification of CAR-T cell activation following lentiviral transduction and co-culture with antigen-positive target cells. These assays support construct validation, specificity testing, and potency assessment across CAR-T development and QC workflows.



TCR/CD3 Effector Cells were transduced with CAR-19 lentivirus and co-cultured with either CD19⁺ Raji target cells or CD19-knockout Raji cells. Luminescence was detected after a 6-hour induction period. CAR-mediated activation was observed only with CD19⁺ targets and was abolished when CD19 was removed, confirming that the assay reflects true CAR:target engagement.

Assessing Lenti-CAR Quality Using Functional CAR Potency Readouts: Luciferase-based T Cell Activation Bioassays provide a sensitive, mechanism-of-action-aligned readout of CAR signaling and can be used to assess the functional potency of lentiviral CAR preparations. The approach enables evaluation of lot quality, degradation effects and manufacturing consistency by quantifying CAR-triggered activation following transduction.



Forced degradation samples of CAR-19 lentivirus were generated by incubating viral preparations at 37°C for 2 – 48 hours. After incubation, samples were used to transduce TCR/CD3 Effector Cells, which were then co-cultured with CD19⁺ target cells. Heat treatment reduced the functional potency of the lentiviral preparations, reflected by increased EC₅₀ values and decreased maximum responses. These data demonstrate that CAR-driven reporter activation can be used as a functional measure of Lenti-CAR quality.

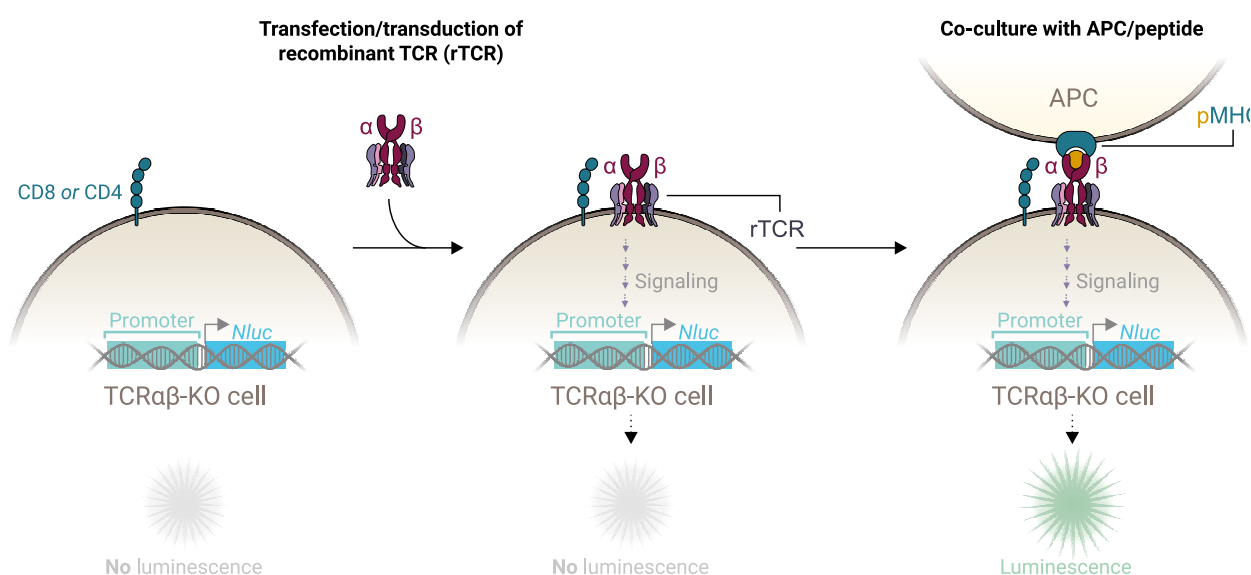
3. Tools for Potency & Functional Analysis

3.3 How Do I Screen and Validate New CARs or TCRs?

T Cell Activation Bioassays (TCR $\alpha\beta$ -KO)

The T Cell Activation Bioassay (TCR $\alpha\beta$ -KO) is a bioluminescent cell-based assay for functional characterization and potency assessment of transgenic TCR constructs. The assay uses a CRISPR/Cas9-engineered Jurkat T-cell line in which endogenous TCR α and TCR β chains are knocked out, eliminating mixed TCR pairing and enabling clean expression of a user-defined TCR. These cells express a TCR pathway-dependent luciferase reporter, providing a quantitative readout of antigen-dependent TCR activation. The assay is available in CD4⁺, CD8⁺, and CD4⁺/CD8⁺ formats to support analysis of MHC I- and MHC II-restricted TCRs and to interrogate CD4/CD8 co-receptor dependence. Transgenic TCRs can be introduced by transfection or lentiviral transduction, and upon co-culture with APCs presenting cognate peptide-MHC, TCR engagement triggers downstream signaling that induces luciferase expression.

Assay Principle



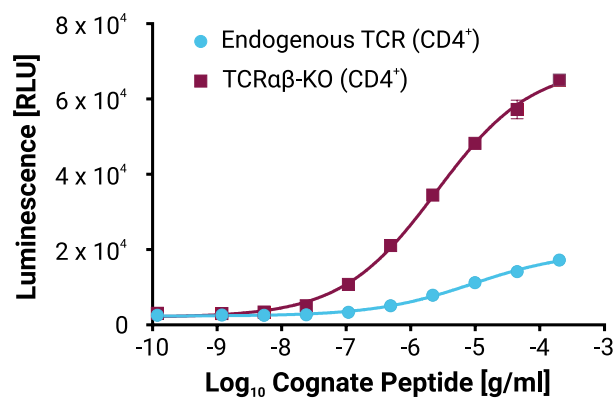
Without a transgenic TCR, TCR $\alpha\beta$ -KO cells do not respond to peptide-MHC stimulation and show minimal luminescence. When a transgenic TCR is introduced via transfection or lentiviral transduction, co-culture with APCs presenting the cognate peptide induces TCR pathway-driven luciferase expression.

Key Benefits of T Cell Activation Bioassays

- **Mechanism-of-action (MOA)-based functional readouts** that reflect true TCR/CAR engagement, downstream signaling, and biologically relevant activation.
- Quantitative, reproducible potency data, pre-qualified according to **ICH guidelines**, enabling robust assay performance suitable for development and QC workflows.

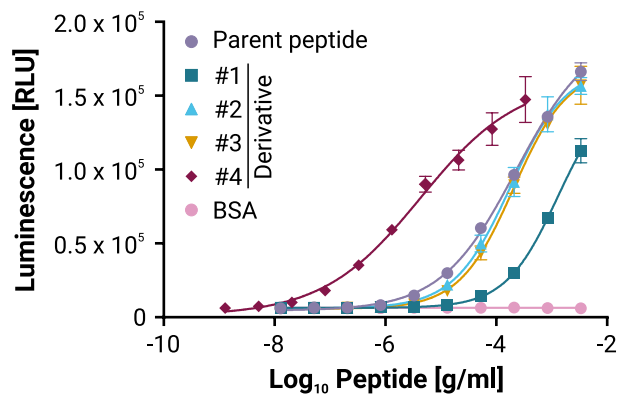
Application Examples:

Transgenic TCR Validation: TCR $\alpha\beta$ -KO Jurkat cells lack endogenous TCR chains and therefore allow highly specific, background-free assessment of transgenic TCR constructs. When engineered to express a user-defined TCR, these cells respond selectively to cognate peptide presented by antigen-presenting cells (APCs), enabling sensitive functional characterization of affinity, specificity, and safety.



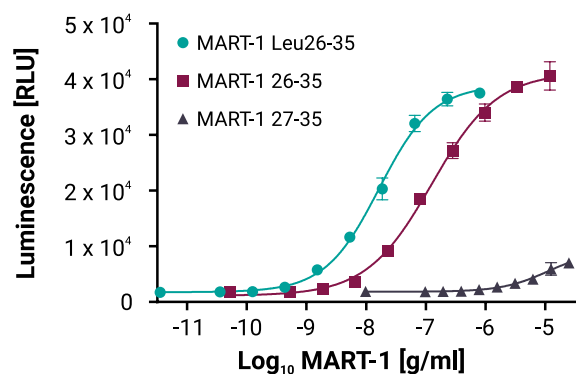
TCR $\alpha\beta$ -KO Enhances Sensitivity to Cognate Peptide.

TCR $\alpha\beta$ -KO (CD4 $^{+}$) cells and parental Jurkat cells expressing the same transgenic TCR were co-cultured with HLA-matched APCs pulsed with titrated cognate peptide. TCR $\alpha\beta$ -KO cells showed markedly higher luminescent responses and lower EC_{50} values compared to parental Jurkat cells, illustrating the benefit of removing endogenous TCR chains and preventing mixed TCR pairing.



Peptide Variant Profiling Using TCR $\alpha\beta$ -KO CD4 $^{+}$ Cells.

TCR $\alpha\beta$ -KO (CD4 $^{+}$) cells expressing an HA-specific transgenic TCR were stimulated with cognate peptide and a panel of peptide derivatives. The assay resolved clear potency differences among the variants, identifying both enhanced and attenuated activators. These data demonstrate the value of the TCR $\alpha\beta$ -KO platform for specificity ranking and early safety assessment of engineered TCR constructs.



CD8 Co-Receptor Enhances Activation of MHC-I-Restricted TCRs.

TCR $\alpha\beta$ -KO (CD8 $^{+}$) cells expressing a MART-1-specific TCR were stimulated with multiple MART-1 peptide variants. Compared with CD4 $^{+}$ cells, CD8 expression increased sensitivity to peptide stimulation, reflected by lower EC_{50} values and higher maximal responses. This demonstrates how different TCR $\alpha\beta$ -KO formats enable evaluation of co-receptor dependence during TCR screening and development.

3. Tools for Potency & Functional Analysis

3.3 How Do I Screen and Validate New CARs or TCRs?

References

Grailer, J. et al. (2023) A novel cell-based luciferase reporter platform for the development and characterization of T-cell redirecting therapies and vaccine development. *J. Immunother.* 46(3):96–106.

Jahan, F. et al. (2023) Using the Jurkat reporter T cell line for evaluating the functionality of novel chimeric antigen receptors. *Front. Mol. Med* 3,1070384.

Nishimura, C.D. et al. (2024) TOP CAR with TMIGD2 as a safe and effective costimulatory domain in CAR cells treating human solid tumors. *Sci Adv.* 10(19).

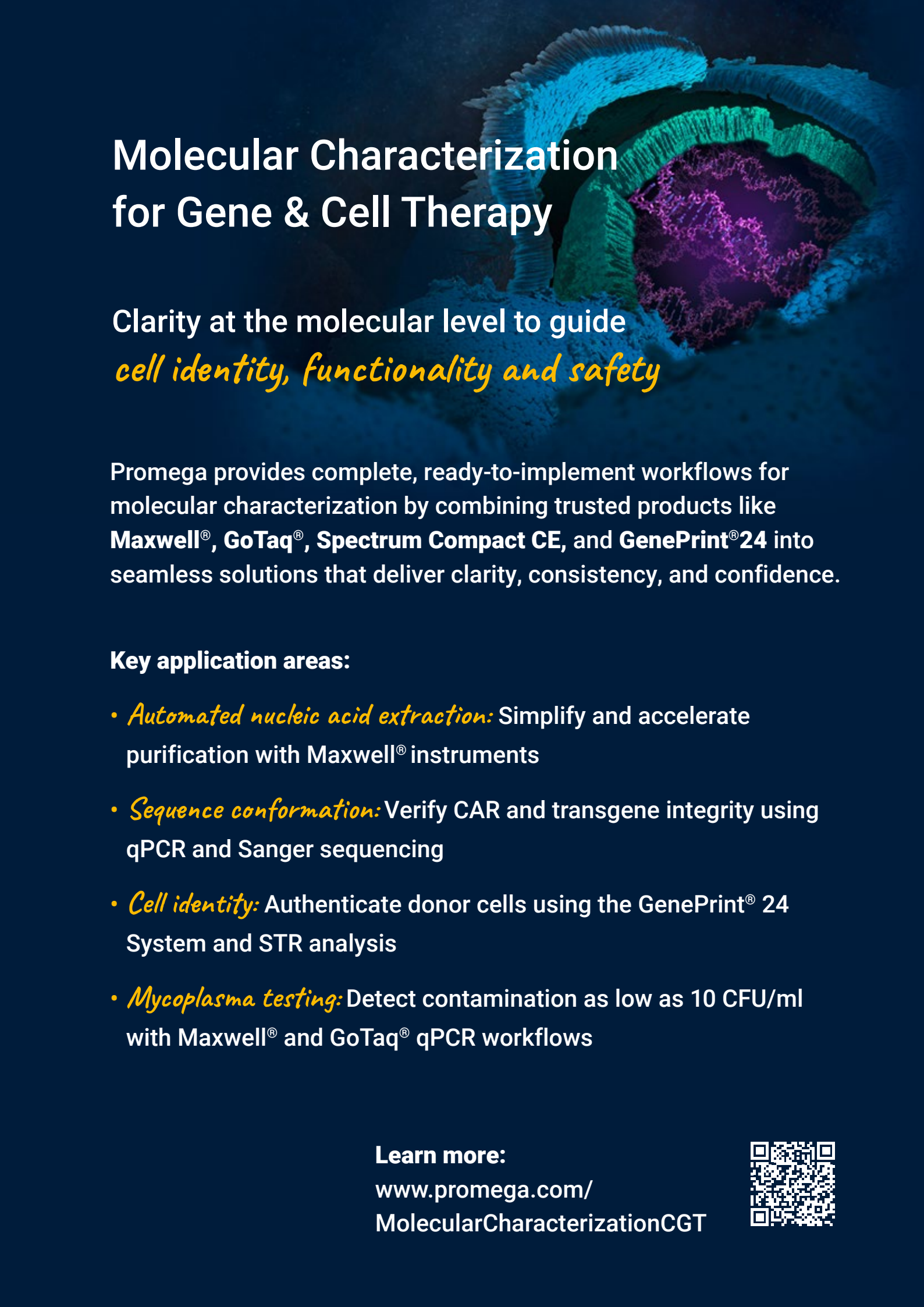
Gilden, J.K. et al. (2025) A bioluminescent reporter bioassay for in-process assessment of chimeric antigen receptor lentiviral vector potency. *Antib. Ther.* 8(1):40–46.

Product Box

T Cell Activation Bioassay (NFAT)	Cat. # J1601
T Cell Activation Bioassay (IL-2)	Cat. # J1631
T Cell Activation Bioassay (TCRαβ-KO, CD4 ⁺)	Cat. # GA1172
T Cell Activation Bioassay (TCRαβ-KO, CD8 ⁺)	Cat. # GA1162
T Cell Activation Bioassay (TCRαβ-KO, CD4 ⁺ , CD8 ⁺)	Cat. # GA1182
Bio-Glo™ Luciferase Assay System	Cat. # G7941
Bio-Glo-NL™ Luciferase Assay System	Cat. # J3081



A more detailed product list can be found on pages 34 – 37.



Molecular Characterization for Gene & Cell Therapy

Clarity at the molecular level to guide
cell identity, functionality and safety

Promega provides complete, ready-to-implement workflows for molecular characterization by combining trusted products like **Maxwell®**, **GoTaq®**, **Spectrum Compact CE**, and **GenePrint® 24** into seamless solutions that deliver clarity, consistency, and confidence.

Key application areas:

- *Automated nucleic acid extraction:* Simplify and accelerate purification with Maxwell® instruments
- *Sequence conformation:* Verify CAR and transgene integrity using qPCR and Sanger sequencing
- *Cell identity:* Authenticate donor cells using the GenePrint® 24 System and STR analysis
- *Mycoplasma testing:* Detect contamination as low as 10 CFU/ml with Maxwell® and GoTaq® qPCR workflows

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[www.promega.com/
MolecularCharacterizationCGT](http://www.promega.com/MolecularCharacterizationCGT)



4. Cell Tracking and Interaction Analysis *in vivo*

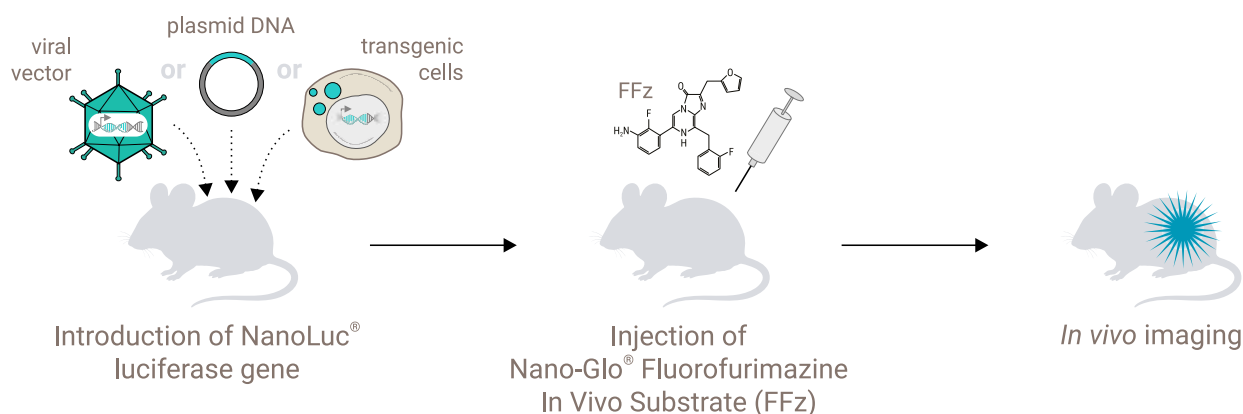
4.1 How can I track cell populations over time in live animals?

Monitoring how CAR-T cells behave and engage tumor cells *in vivo* is key to advancing cell therapy research. The Nano-Glo® In Vivo Substrates, Fluorofurimazine (FFz) enables bright, stable, and noninvasive bioluminescent imaging of NanoLuc® luciferase-expressing cells in living organisms. FFz supports sensitive, long-lasting signal detection for tracking CAR-T cell activation, migration, and persistence in peripheral tissues to enable comprehensive, real-time monitoring of CAR-T and tumor cell dynamics throughout the body.

Nano-Glo® In Vivo Substrates: Fluorofurimazine, FFz

Nano-Glo® Fluorofurimazine In Vivo Substrate (FFz) is an optimized, aqueous-soluble substrate for *in vivo* detection of NanoLuc® luciferase, NanoLuc® fusion proteins, or reconstituted NanoBiT® luciferase. It offers high substrate bioavailability, bright, stable signals, and flexible delivery options like intravenous (IV) or intraperitoneal (IP) injection.

Assay Principle



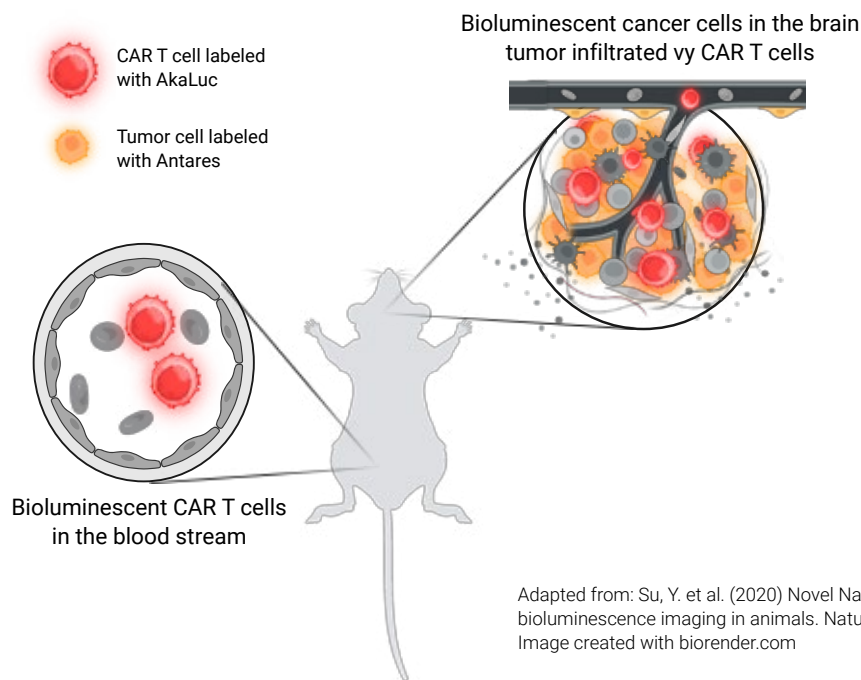
Nano-Glo® In Vivo Substrates enable noninvasive bioluminescent imaging of NanoLuc®-expressing cells in living animals. Signals are detected using an *in vivo* imaging system (IVIS), allowing longitudinal analysis of cellular localization, expansion, and persistence without phototoxic effects.

NanoLuc® is a small 19 kDa luciferase emitting blue light (~450nm). To overcome heme absorption, red-shifted BRET reporters such as Antares were developed, enabling improved deep-tissue imaging with emission peaks around 600 nm. Antares-expressing mouse models and compatible expression vectors are available through specialized repositories, Promega, or Addgene. An Antares reporter mouse model is commercially available through The Jackson Laboratory (RRID:IMSR_JAX:038889).

NanoLuc® reporters can also be combined with firefly luciferase-based systems such as AkaLuc for dual-reporter imaging applications.

Application Examples:

Long term imaging of CAR-T- and cancer cell interaction *in vivo*



CAR-T cells were labeled with AkaLuc (red signal) and transplanted tumor cells with Antares (NanoLuc–CyOFP1, orange signal). This dual-luciferase system enabled longitudinal imaging of both populations *in vivo*. Tumors regressed and CAR-T cells accumulated at the tumor site when matching CAR-T cells were injected, whereas tumors continued to grow and mock CAR-T cells showed no tumor accumulation.

References

Theruvath, J. et al. (2020) Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors. *Nat. Med.* 26, 712–9.

Wang, T. et al. (2023) Visualizing cell-cell communication using synthetic notch activated MRI. *Proc. Natl. Acad. Sci.* 120, e2216901120.

Product Box

Nano-Glo® Fluorofurimazine In Vivo Substrate (FFz)

Cat. # N4100



A more detailed product list can be found on pages 34 – 37.

5. STR Genotyping in Cell Therapy Quality Control

5.1 How to use STR genotyping for quality control in cell therapy?

In CAR-T and TCR-T cell manufacturing, maintaining the genetic identity of the therapeutic cells is essential for patient safety and regulatory compliance. STR genotyping provides a precise DNA-based method to confirm donor-product matching, detect cross-contamination, and ensure the authenticity of both autologous and allogeneic cell products.

GenePrint® 24 System – Confidence for Cell Line Identity and Mixed Sample Analysis

The **GenePrint® 24 System**, originally developed by Promega for human cell-line authentication and forensic identification, provides a robust and regulatory-aligned STR genotyping platform that is increasingly applied in cell-therapy quality-control workflows.

The system enables multiplex amplification and fluorescent detection of 24 highly informative loci, generating a detailed and reproducible genetic profile for human cell identification.

Workflow for cell authentication by DNA Fingerprinting



Human cell line authentication for cell therapy by DNA Fingerprinting. **1.** Using the **Maxwell® RSC 48 instrument**, gDNA can be purified from cell lines, alternatively is possible to perform direct amplification for cells spotted on storage cards, **2.** amplified using the **GenePrint® 24 System**, **3.** fragments analyzed by capillary electrophoresis using the **Spectrum Compact CE System**, and **4.** analyzed using **GeneMarkerHID®** for Spectrum.

Application Example

CAR-T Donor Authentication using GenePrint® 24 System

Unstimulated T Cells and finished CAR-T product were spotted on FTA cards using direct amplification of the **GenePrint® 24 System** and analyzed on a **Spectrum Compact CE System**, shows 100 % concordance of allele calls within donors.

	Donor 2006		Donor 7001	
	T cells	CAR-T	T cells	CAR-T
AMEL	XY	XY	XX	XX
D3S1358	15	15	17,18	17,18
D1S1656	14,16	14,16	15,17	15,17
D2S441	10	10	10,11	10,11
D10S1248	13,14	13,14	13,14	13,14
D13S317	12,14	12,14	12,13	12,13
Penta E	12,16	12,16	10,12	10,12
D16S539	11,12	11,12	11,12	11,12
D18S51	12	12	12,16	12,16
D2S1338	22,23	22,23	16,17	16,17
CSF1PO	12	12	12	12
Penta D	13,14	13,14	9,13	9,13
TH01	6,9	6,9	8	8
vWA	18	18	16	16
D21S11	28,32.2	28,32.2	28,3	28,3
D7S820	12	12	8,12	8,12
D5S818	11	11	11,13	11,13
TPOX	8,11	8,11	8,9	8,9
DYS391	11	11	N/A	N/A
D8S1179	14,15	14,15	12	12
D12S391	17,2	17,2	17,18	17,18
D19S433	13	13	14,16	14,16
FGA	19,26	19,26	24	24
D22S1045	15	15	14,15	14,15

Note: STR analysis confirms cell identity and detects contamination events. It does not assess clonal drift or genomic instability, which should be evaluated using complementary molecular or cytogenetic assays as part of a comprehensive cell therapy quality control strategy.

Product Box

Maxwell® RSC 48 Instrument	Cat. # AS8500
Maxwell® RSC Genomic DNA Kit 48 preps	Cat. # AS1880
GenePrint® 24 System 100 rxn	Cat. # B1870
Spectrum Compact CE System, 4-Capillary	Cat. # CE1304
Spectrum CE System, 8-Capillary	Cat. # CE1008



A more detailed product list can be found on pages 34 – 37.

6. Ordering Information

Cell Health Assays

Product	Size	Cat. #
CellTiter-Glo® Luminescent Cell Viability Assay	10, 100 ml, 10x10 ml or 10x100 ml	G7570, G7572, G7571, G7573
CellTiter-Glo® 2.0 Cell Viability Assay	10, 100 or 500 ml	G9241, G9242, G9243
CellTiter-Glo® 3D Cell Viability Assay	10, 100 ml or 10x10 ml	G9681, G9683, G9682
Lumit® Cell Proliferation Assay (Human Ki-67)	100, 500 or 1000 assays	GC1000, GC1002, GC1001
LDH-Glo™ Cytotoxicity Assay	10 or 50 ml	J2380, J2381

Metabolism Assays

Product	Size	Cat. #
Glucose Uptake-Glo™ Assay	5, 10 or 50 ml	J1341, J1242, J1343
Glucose-Glo™ Assay	5 or 50 ml	J6021, J6022
Lactate-Glo™ Assay	5 or 50 ml	J5021, J5022
Glycogen-Glo™ Assay	5 or 50 ml	J5051, J5052
Pyruvate-Glo™ Assay	5 or 50 ml	J4051, J4052
Malate-Glo™ Assay	5 or 50 ml	JE9100, JE9200
Glutamine/Glutamate-Glo™ Assay	5 or 50 ml	J8021, J8022
BCAA-Glo™ Assay	5 or 50 ml	JE9300, JE9400
Triglyceride-Glo™ Assay	5 or 50 ml	J3160, J3161
Cholesterol/Cholesterol Ester-Glo™ Assay	5 or 50 ml	J3190, J3191
BHB-Glo™ Assay	5 or 50 ml	JE9500, JE9600
ROS-Glo™ H ₂ O ₂ Assay	10 or 50 ml	G8820, G8821
NADP/NADPH-Glo™ Assay	10 or 50 ml	G9081, G9082
NAD/NADH-Glo™ Assay	10 or 50 ml	G9071, G9072

Lumit® Cytokine Immunoassays

Product	Size	Cat. #
Lumit® IL-1 β (Human) Immunoassay	100, 500 or 1000 assays	W6010, W6012, W6011
Lumit® IL-2 (Human) Immunoassay	100, 500, or 1000 assays	W6020, W6022, W6021
Lumit® IL-4 (Human) Immunoassay	100, 500, or 1000 assays	W6060, W6062, W6061
Lumit® IL-6 Human Immunoassay	100, 500, or 1000 assays	W6030, W6032, W6031
Lumit® IL-8 (Human) Immunoassay	100, 500, or 1000 assays	W1460, W1462, W1461
Lumit® IL-10 (Human) Immunoassay	100, 500, or 1000 assays	W6070, W6072, W6071
Lumit® IL-12 p70 (Human) Immunoassay	100, 500, or 1000 assays	W1850, W1852, W1851
Lumit® IL-17A (Human) Immunoassay	100, 500, or 1000 assays	W1430, W1432, W1431
Lumit® Active IL-18 (Human) Immunoassay	100, 500, or 1000 assays	W1910, W1912, W1911
Lumit® VEGF-A (Human) Immunoassay	100, 500, or 1000 assays	W1880, W1882, W1881
Lumit® IFN- β (Human) Immunoassay	100, 500, or 1000 assays	W1810, W1812, W1811
Lumit® IFN- γ (Human) Immunoassay	100, 500, or 1000 assays	W6040, W6042, W6041
Lumit® TNF- α (Human) Immunoassay	100, 500, or 1000 assays	W6050, W6052, W6051

HiBiT Target Cell Killing Bioassays

Product	Size	Cat. #
ViaScript® (HiBiT) TCK Bioassay	1x or 5x kit	CS3628B19*, CS3628B21*
HaloTag®-HiBiT Vector [CAG / BlastR]	20 μ g	CS1956B17*
HaloTag®-HiBiT-LentiB4 Transfer Vector [CMV / BlastR]	20 μ g	CS3055A56*
Ramos (HiBiT) TCK Bioassay	1x or 5x kit	JA1411, JA1415
H929 (HiBiT) TCK Bioassay	1x or 5x kit	JA1511, JA1515
Raji (HT-HiBiT) TCK Bioassay	1x or 5x kit	JA1211, JA1215
Raji (HT-HiBiT) CD19-KO TCK Bioassay	1x or 5x kit	JA1251, JA1255

* CS product numbers indicate **Early Access (EA) Products** and cannot be found in the online catalog. If you need more information, please contact us.

Please note that there is **no guarantee** that CS products will be added to the catalog. **Protocols and components are subject to change**, and CS products **may be discontinued without prior notice**.

6. Ordering Information

HiBiT Target Cell Killing Bioassays (continued)

Product	Size	Cat. #
Raji (HT-HiBiT) CD19/20-KO TCK Bioassay	1x or 5x kit	JA1311, JA1215
Raji (LDH-HiBiT) TCK Bioassay	1x or 5x kit	JA1311, JA1315
Raji (LDH-HiBiT) CD19-KO TCK Bioassay	1x or 5x kit	JA1221, JA1225
Raji (LDH-HiBiT) CD20-KO TCK Bioassay	1x or 5x kit	JA1231, JA1235
Raji (LDH-HiBiT) CD19/20-KO TCK Bioassay	1x or 5x kit	JA1241, JA1245

All TCK Bioassays include the following components: Fetal Bovine Serum, RPMI 1640 Medium, Bio-Glo-NB TCK Luciferase Assay Substrate, Bio-Glo-NB TCK Luciferase Assay Buffer, Bio-Glo-NB TCK LgBiT Protein.

This table shows a selection of available TCK Bioassays. Depending on the cell line, additional assays and formats, including Cell Propagation Models (CPM) and Cell Banks, may be available. **For the complete, current list, including Early Access products, please contact Promega or your local sales representative.**

T Cell Activation Bioassays

Product	Size	Cat. #
T Cell Activation Bioassay (NFAT)	1x, 5x kit, CMP	J1621, J1625, J1601
T Cell Activation Bioassay (IL-2)	1x, 5x kit, CMP	J1651, J1655, J1631
T Cell Activation Bioassay (TCR $\alpha\beta$ -KO, CD4 ⁺)	CPM, Cell Bank	GA1172, GA1210
T Cell Activation Bioassay (TCR $\alpha\beta$ -KO, CD8 ⁺)	CPM, Cell Bank	GA1162; GA1220
T Cell Activation Bioassay (TCR $\alpha\beta$ -KO, CD4 ⁺ , CD8 ⁺)	CPM, Cell Bank	GA1182, GA1230
T Cell Activation Bioassay (NF κ B)	CPM	CS1979A01*
T Cell Activation Bioassay (NFAT/ NF κ B)	CPM	CS1978B04*
Bio-Glo™ Luciferase Assay System	10 ml, 100 ml	G7941, G7940
Bio-Glo-NL™ Luciferase Assay System	10 ml, 100 ml, 10x100 ml	J3081, J3082, J3083

CPM – Cell propagation model

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Please note that there is **no guarantee** that CS products will be added to the catalog. **Protocols and components are subject to change**, and CS products **may be discontinued without prior notice**.

Cell Tracking & Interaction Analysis *in vivo*

Product	Size	Cat. #
Nano-Glo® Fluorofurimazine In Vivo Substrate (FFZ)	1x or 5x	N4100, N4110

STR Genotyping

Product	Size	Cat. #
GenePrint® 24 System	100 rxn	B1870
GenePrint® 24 System	400 rxn	B1874
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC Genomic DNA Kit	48 preps	AS1880
Spectrum Compact CE System	1 each	CE1304

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7. Contacts

100+

Countries

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