# nCounter® CAR-T Characterization Panel

Gene Expression Panel

CAR-T Development • Manufacturing • Pre and Post Infusion Analysis

Confidently profile CAR-T products throughout your manufacturing process with a highly reproducible and automated assay. Created in collaboration with experts in CAR-T therapy, the nCounter CAR-T Characterization Panel measures eight critical components of CAR-T biology and facilitates the development of robust product release assays with a streamlined workflow that potentially reduces vein to vein time.
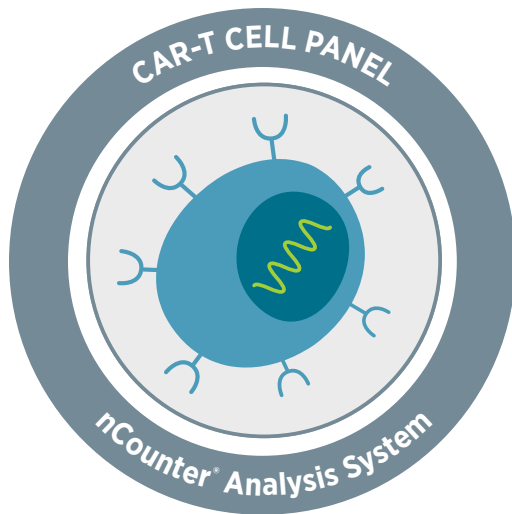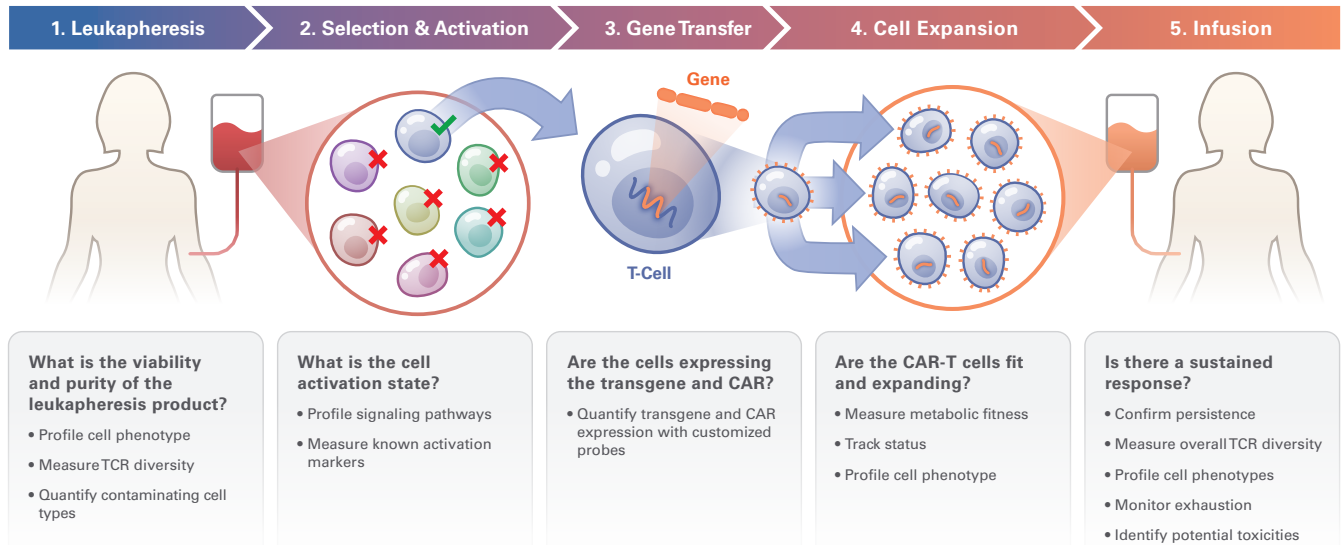
Product Highlights

- Expertly curated content includes 780 genes covering T-cell biology

  - Signaling pathways influencing T-cell differentiation and activity

  - Phenotypic and metabolic switches

  - T-cell subsets associated with differentiation and activation state

  - T-Cell Receptor (TCR) diversity

  - Immune cell profiling

- Confirm transgene insertion and CAR expression with Panel Plus

- nCounter workflow is streamlined, user-friendly, and efficient with just 15 minutes total hands-on time

Feature	Specifications
Number of Targets	780 (Human), Including internal reference genes
Sample Input - Standard (No amplification required)	25-300 ng
Sample Input - Low Input	As little as 1 ng with nCounter Low Input Kit (sold separately)
Sample Type(s)	Sorted T-cells, CAR-T cells, CAR-T Manufacturing Product, PBMCs, whole blood, FFPE-derived RNA, total RNA, fragmented RNA, cell lysate
Customizable	Add up to 55 unique genes with Panel-Plus
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software (RUO), and ROSALIND® Platform

CAR-T Therapy Workflow

Understanding each step of the CAR-T workflow is critical to ensuring quality and efficacy of the final CAR-T product. The nCounter CAR-T Characterization Panel can be used throughout development and manufacturing as a standardized panel of genes for optimizing methods, developing manufacturing acceptance criteria, as well as understanding the host influences beyond manufacturing.

1. Leukapheresis	2. Selection & Activation	3. Gene Transfer	4. Cell Expansion	5. Infusion
What is the viability and purity of the leukapheresis product? • Profile cell phenotype • Measure TCR diversity • Quantify contaminating cell types	**What is the cell activation state?** • Profile signaling pathways • Measure known activation markers	**Are the cells expressing the transgene and CAR?** • Quantify transgene and CAR expression with customized probes	**Are the CAR-T cells fit and expanding?** • Measure metabolic fitness • Track status • Profile cell phenotype	**Is there a sustained response?** • Confirm persistence • Measure overall TCR diversity • Profile cell phenotypes • Monitor exhaustion • Identify potential toxicities

Immune Cell Profiling and TCR Diversity Features

Genes included in the CAR-T Characterization Panel provide unique cell profiling and TCR diversity data to measure the relative abundance of immune cell types[1] and shifts in TCR populations[2]. The tables below summarize the cell type and TCR diversity content in the panel, as qualified through biostatistical approaches and selected literature in the field of immunology.

Relative Cell Type Abundance

Cell Type	Associated Human Genes
B cells	9
CD45	1
CD8 T-cells	2
Cytotoxic cells	10
Dendritic cells	3
Exhausted CD8	4
Macrophages	4
Mast-cells	4
Neutrophils	6
NK CD56dim cells	3
NK cells	2
T-cells	6
Th1 cells	1
Treg	1

TCR Diversity Content

Chain Type	Constant Chains	Variable Chains
Alpha	TRAC	TRAV_ 45 probes, 46 genes TRAV8-2 and 8-4 covered by 1 probe
Beta	TRBC1/2	TRBV_ 46 probes, 48 genes TRBV6-3 and 6-2 covered by 1 probe TRBV12-4 and 12-3 covered by 1 probe
Gamma	TRAC	TRGV_ 5 probes, 6 genes TRGV3 and 5 covered by 1 probe
Delta	TRDC	TRDV_ 3 probes, 3 genes

Immune Cell Markers

CD3D/E/G, CD4, CD8A/B, PTPRC (CD45), CD45R0, CD45RA, SELL (CD62L), CCR7, CD28, CD40LG, IL2RA (CD25), NCR1 (NKp46)

1. Danaher P. et al. Gene expression markers of Tumor Infiltrating Leukocytes JITC 2017
2. Zhang M. et al. A new approach to simultaneously quantify both TCR α and β-chain diversity after adoptive immunotherapy Clin Cancer Res 2012

CAR-T Characterization Panel Functional Annotations

Essential CAR-T Biology	Description	Pathways and Processes
Phenotype	Multiple subtypes of T-cells and the phenotypic changes that accompany them can be distinguished via the cytokines and pathways that maintain, promote, and modulate their activity.	Notch, Wnt signaling, Tfh, TGF-beta, Th1, Th17, Th2, Th9, Treg, Innate-like T-cells, Vitamin A (RA) Signaling
Cell Types	Other cell types can contaminate the population of active CAR-T cells. Similarly, measurement of B-cell populations may aid in assessment of whole blood post-infusion measurement of tumor burden in B-cell lymphomas.	Immune cell profiling
TCR Diversity	The TCR diversity of CAR-T cells can provide information on the number of clones present after leukapheresis, manufacturing, and infusion.	TCR Content
Activation	T-cell activation is primarily mediated by antigens presented to the TCR complex and modulated by costimulatory molecules. Downstream of the TCR, multiple pathways induce transcriptional changes that lead to the production of chemokines and cytokines. Cell surface receptors signal a change in phenotype.	Chemokine Signaling, Costimulatory Molecules, Interleukin Signaling, TCR signaling, JAK-STAT, MAPK and PI3K Signaling, Myc targets, NFAT, Antigen processing & presentation, T-cell activation markers
Metabolism	Fundamental changes in T-cell metabolism are induced upon activation to enable rapid cell division and thus expansion of relevant clones. These changes can be observed across basic metabolic pathways, including carbohydrate and fatty acid metabolism.	Glycolysis, Mitochondrial biogenesis, Fatty Acid Metabolism, Glutamine metabolism, Circadian Clock, One-carbon metabolism, Oxidative phosphorylation, mTOR, Cell Cycle, Autophagy
Persistence	Ongoing presence of a T-cell and cytotoxic T-cell population can be measured via cell type profiling. By measuring molecules involved in T-cell migration we can assess the ability of T-cells to home in on their target antigens.	T-cell migration, T-cell type profiling
Exhaustion	T-cell exhaustion can be induced by costimulatory molecules, other cell-cell interactions, and cell death via apoptosis.	T-cell exhaustion markers, Apoptosis, Interactions with Non-Lymphoid Cells, Costimulatory Molecules
Toxicity	Toxicity is correlated with certain cytokine and chemokine profiles. These signals can induce a pro-inflammatory environment in various tissues and lead to off-target toxicities of CAR-T treatment.	NK cell cytotoxicity, NKT Receptors, NF-κB, Type I interferon signaling, Type II interferon signaling, Interleukin signaling, Chemokine signaling

nSolver™ Analysis Software

NanoString offers advanced software tools that address the continuous demands of data analysis and the need to get simple answers to specific biological questions easily. Genes included in the CAR-T Characterization panel are organized and linked to various advanced analysis modules to allow for efficient analysis of the eight essential aspects of CAR-T biology in addition to the standard nSolver analysis.

Advanced Analysis Modules available for CAR-T Characterization:

- Normalization
- Quality Control
- Pathway Analysis
- Cell Profiling
- Differential Expression
- Gene Set Analysis
- Built-in compatibility for Panel Plus and Protein analysis

ROSALIND

ROSALIND is a cloud-based platform that enables scientists to analyze and interpret differential gene expression data without the need for bioinformatics or programming skills. ROSALIND makes analysis of nCounter data easy, with guided modules for:

- Normalization
- Quality Control
- Individual Pathway Analysis
- Differential Expression
- Gene Set Analysis

nCounter customers can access ROSALIND at rosalind.bio/nanostring

TCR Diversity Report

The TCR Diversity report is available as an a la carte purchase within ROSALIND and evaluates the expression of variable regions (alpha, beta, gamma, and delta) of the T cell receptor. The variable regions are assessed for overall expression (above or below background) and normalized to a panel standard which allows for more precise quantification of these variable regions. An estimate of TCR Diversity is calculated with the T cell receptor score.

The TCR score calculates the diversity of T cell receptor beta variable regions within a sample. The score is based on the Shannon Diversity index calculation, a mathematical measure of species diversity within a community. This ecological calculation accounts for the abundance and evenness of the variable regions present within a given sample versus the population of T cell receptors within a given dataset. A given score is relative within a dataset, and a higher TCR score means there is a more diverse population of variable regions or a less clonal population. A lower TCR score means there is less diversity or a more clonal population. True clonality can only be determined by full sequencing of the T cell receptors, but clonality can be estimated by measuring the diversity of TCR beta variable regions.

Summary	Heatmap	TCR Score
Plots for number of probes detected above background, broken down by variable regions and grouping variables selected for analysis.	Heatmaps of the probes detected above background and the panel standard normalized heatmaps broken down by variable regions.	The TCR score for each sample is shown in different ways depending on the type of grouping variable within the analysis.
An explanation of the report and relevant assay QC information displayed in a table.		
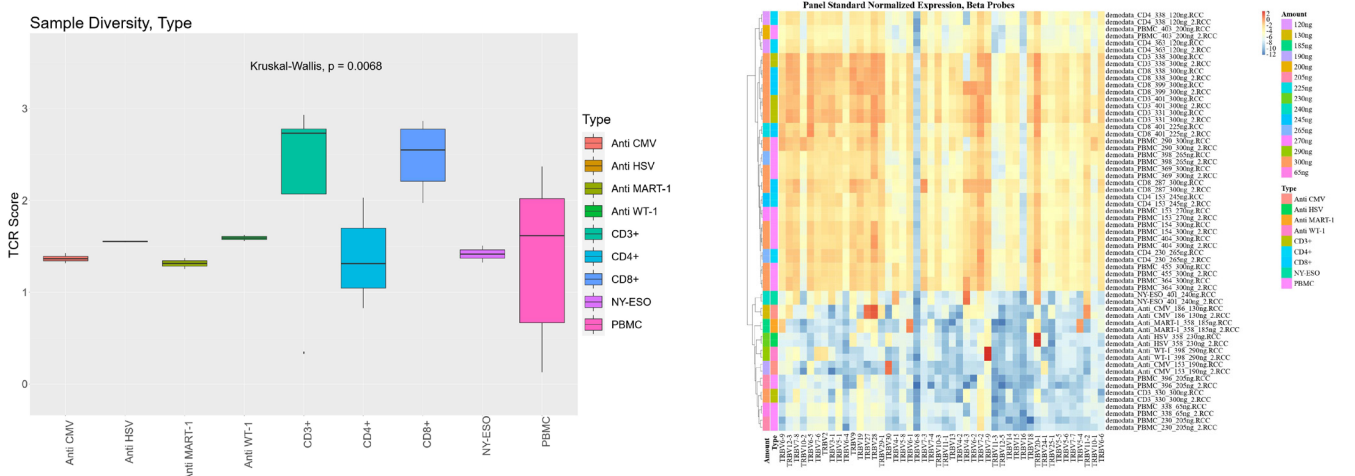
Quantify Transgene Insertion and CAR Expression

Confirm transgene insertion and CAR expression with customizable solutions that work with the CAR-T Characterization panel. Spike-in up to 55 custom RNA targets with the Panel Plus product. Because the nCounter platform is analyte agnostic, you can evaluate transgene insertion and CAR expression simultaneously with one run on a single sample.

Ordering Information

Gene Expression Panels arrive ready-to-use and generally ship within 24 hours following purchase.

Product	Product Description	Quantity	Catalog Number
nCounter Human CAR-T Characterization Panel	Gene Expression CodeSet measuring eight essential components of CAR-T biology with 780 genes. No Master Kit	12 Reactions	XT-CSO-HCART1-12
nCounter CAR-T Characterization Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the 770 unique probe targets in the panel including TCR diversity.	12 Reactions	PSTD-HCART-12
nCounter Human CAR-T Panel Primer Pool	Low input protocol and primer designs available.	N/A	Ask Your Sales Rep
TCR Diversity Data Analysis Report	ROSALIND data analysis report for the CAR-T Characterization Panel. Minimum purchase of 12 samples.	1 sample	ROSA-TCRDIV-1
Low RNA Input Kit	Kit for use with low input protocol; primer designs available.	48 Reactions	LOW-RNA-48
nCounter Analysis System Master Kit Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing on nCounter Analysis System	12 Reactions	NAA-AKIT-012
nCounter SPRINT Cartridge 1 Cartridge, 12 lanes	Sample Cartridge for nCounter SPRINT System	12 Reactions	SPRINT-CAR-1.0
nCounter SPRINT Reagent Pack	nCounter SPRINT Reagent Pack containing Reagents A, B, C, and Hybridization Buffer	192 Reactions	SPRINT-REAG-KIT

Selected Panel References

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2. Fraietta JA et al. Determinants of Response and Resistance to CD19 Chimeric Antigen Receptor (CAR) T-Cell Therapy of Chronic Lymphocytic Leukemia. Nature Medicine. 2018;24(5):563-71.

3. Raud B et al. Fatty Acid Metabolism in CD8+ T-Cell Memory: Challenging Current Concepts. Immunological Reviews. 2018;283(1):213-31.

4. Vargas TR and Apetoh L. The Secrets of T-Cell Polarization. In: Zitvogel L and Kroemer G. (eds) Oncoimmunology. 2018:69-95.

5. Xiao, X et al. Mucosal-Associated Invariant T-Cells: New Insights into Antigen Recognition and Activation. Frontiers in Immunology. 2017;8:1540.

6. Simone MD et al. Transcriptional Landscape of Human Tissue Lymphocytes Unveils Uniqueness of Tumor Infiltrating T Regulatory Cells. Immunity. 2016;45(5):1135-47.

7. Buck, MD et al. T-Cell Metabolism Drives Immunity. Journal of Experimental Medicine. 2015;212;(9):1345-60.

8. Eberl G et al. Innate Lymphoid Cells: A New Paradigm in Immunology. Science. 2015;348(6237).

9. Best JA et al. Transcriptional Insights into the CD8+ T-Cell Response to Infection and Memory T-Cell Formation. Nature Immunology. 2013;14(4):404-12.

10. Chtanova T et al. Identification of T-Cell-Restricted Genes, and Signatures for Different T-Cell Responses, Using a Comprehensive Collection of Microarray Datasets. Journal of Immunology. 2005;175(12):7837-47.

For more information, please visit nanostring.com/CAR-T

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