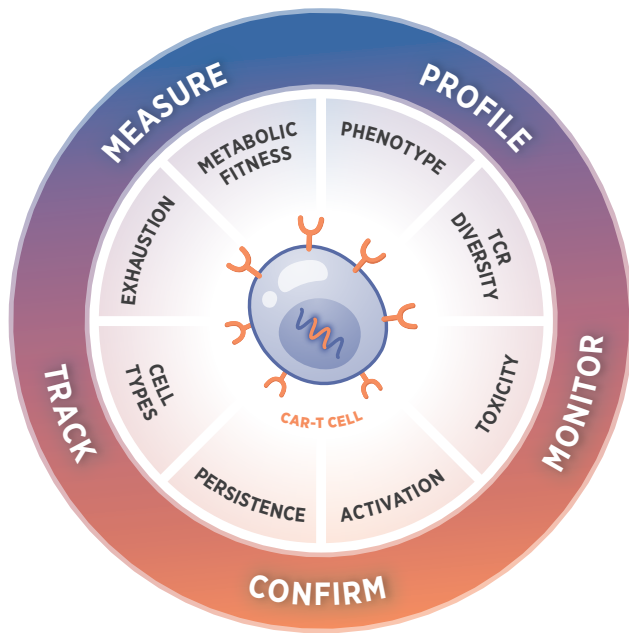


# nCounter® CAR-T Characterization Panel

## 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.



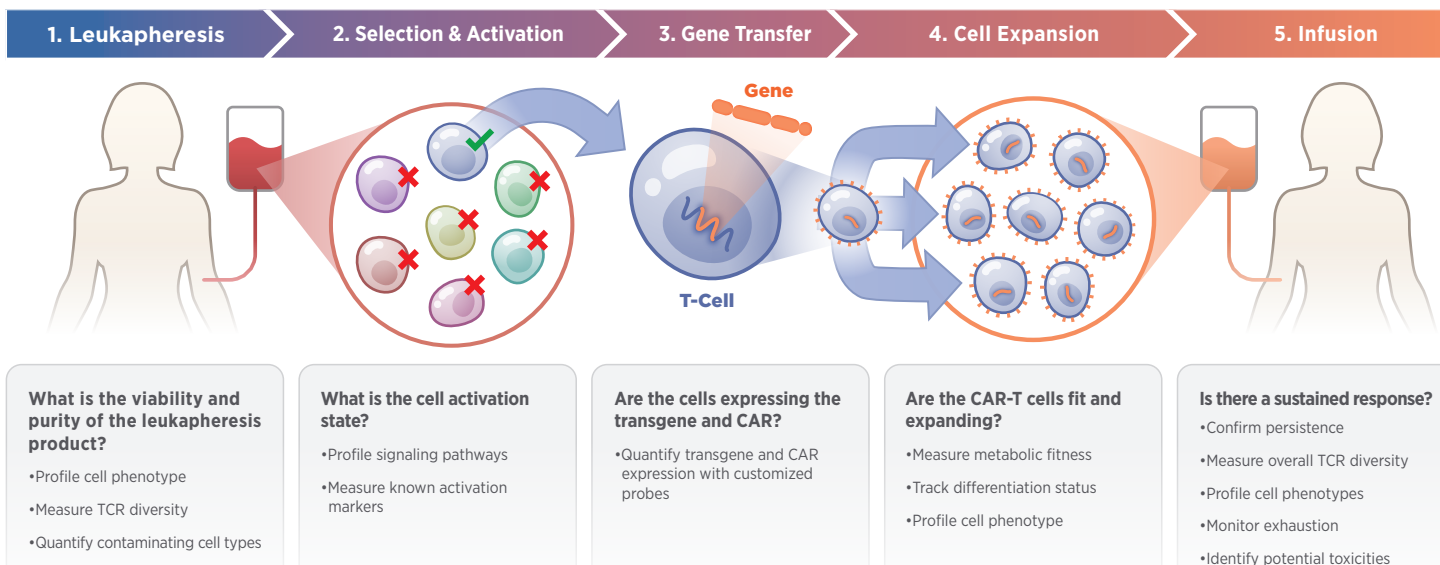
### Product Highlights

- 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 and Vantage 3D™ Protein Barcoding Service
- 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 RNA 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
Customization	Add up to 55 unique genes with Panel-Plus and up to 10 custom protein targets
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software (RUO)

## 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.



## 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<sup>1</sup> and shifts in TCR populations<sup>2</sup>. 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
- TCR Diversity Score (Coming Soon)
- Differential Expression
- Gene Set Analysis
- Built-in compatibility for Panel Plus and Protein analysis

## 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 30 custom RNA targets with the Panel Plus product or create custom protein assays for quantifying CAR expression by providing up to 10 antibodies for our Vantage 3D™ Protein Barcoding Service. Because the nCounter platform is analyte agnostic, you can evaluate transgene insertion and CAR expression simultaneously with one run on a single sample.

To view the annotated gene lists for the CAR-T Characterization Panel, visit: [www.nanostring.com/car-t](http://www.nanostring.com/car-t)

## Ordering Information

Product	Product Description	Quantity	Catalog Number
nCounter Human CAR-T Characterization Panel	Includes 780 genes; 10 internal reference genes for data normalization	12 Reactions	XT-CSO-CART1-12
nCounter Human PanCancer IO 360™ + Panel Standard	Includes 770 genes; 20 internal reference genes for data normalization. Panel standard includes a pool of synthetic oligonucleotides that correspond to the target sequences in the panel	12 Reactions	XT-CSPS-HIO360-12
nCounter Human PanCancer Immune Profiling Panel	Includes 770 genes; 40 internal reference genes for data normalization	12 Reactions	XT-CSO-HIP1-12
nCounter Master Kit (Max and FLEX Systems) Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing on nCounter MAX and FLEX Systems	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

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### NanoString Technologies, Inc.

530 Fairview Avenue North  
Seattle, Washington 98109

### CONTACT US

info@nanostring.com  
Tel: (888) 358-6266  
Fax: (206) 378-6288  
[www.nanostring.com](http://www.nanostring.com)

### SALES CONTACTS

United States: [us.sales@nanostring.com](mailto:us.sales@nanostring.com)  
EMEA: [europe.sales@nanostring.com](mailto:europe.sales@nanostring.com)  
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