nCounter® TCR Diversity Panel

Gene Expression Panel

Biomarker Development • Immune Profiling • Screening

Rapidly determine usage of T cell receptor (TCR) variable regions and shifts in TCR diversity with a novel assay that directly detects variable receptor regions in response to cancer, infectious disease, autoimmunity, or transplanted organs without any enzymatic steps or sequencing. Profile overnight the abundance of alpha, beta, delta, and gamma variable regions as well as constant regions with minimal hands-on time and receive in minutes an automated report with a diversity score for each sample.



Product Highlights

- Directly profile 129 human genes, including T cell receptor variable and constant regions and other T cell markers:
 - Alpha, beta, gamma, and delta variable regions
 - Constant regions
 - CD3/CD4/CD8
- Measure shifts in TCR diversity in response to disease or therapeutic treatments.
- Simultaneously measure TCR diversity and T cell phenotype with a streamlined workflow
- Obtain a report in minutes with a TCR diversity score for each sample
- Customize with up to 55 gene Panel Plus to add additional T cell biology content

Feature	Specifications
Number of Targets	129 (Human), including 10 internal reference genes
Sample Input - Standard (No amplification required)	150-500 ng*
Sample Type(s)	Cultured cells/cell lysates, sorted cells, FFPE-derived RNA, total RNA, fragmented RNA, PBMCs, and whole blood/plasma
Customizable	Add up to 55 unique genes with Panel Plus
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software and the ROSALIND® Platform

*The nCounter TCR Diversity Panel requires users to consider the estimated fraction of T cells likely present in their sample and adjust input amounts accordingly.

For PBMCs or Sorted T cells where T cells are more abundant a minimum of 150 ng is recommended. When sample is not limiting, 300 ng is a more ideal input quantity. For solid tissue or non-immune tissues where the fraction of T cells is typically below 50% a minimum of 500 ng is recommended.

For immune privileged or cold tumor tissues, optimization is always recommended, examples of this include glioblastoma, breast cancer tissue, etc. where samples are likely to have a low fraction of T cells.

Input material can be optimized by scaling to the TRBC1/2 probes with acceptable assay conditions achieved when 2,500 counts are reached with this probe but before saturation is reached for the cartridge.

Input amounts for this panel can be increased from the minimum significantly without saturating the cartridge, although at high input values this may still be possible. Consult with a NanoString Field Application Scientist for any questions related to input amounts and assay optimization.

Application Areas

A diverse population of T cell receptors (TCR) is a hallmark of a healthy, unchallenged adaptive immune system; however, the usage of TCR variable regions changes upon challenge with a pathogen, cancer, or transplanted organ. Shifts in TCR diversity can be indicative of disease onset and progression, response/non-response to treatment, and/or disease recovery. The TCR Diversity Panel has broad applicability for biomarker development, immune profiling, and cell screening across cancer, infectious disease, autoimmunity, and organ transplant.

	Biomarker Development	Immune Profiling	Cell Screening
Questions Addressed with the TCR Diversity Panel	 How does usage of TCR variable regions correlate with disease severity and/or progression? 	 How is the adaptive immune response at the level of the TCR involved in response/ resolution to disease? 	 Which cell populations have novel TCR fingerprints worthy of deep sequencing?
	• How does usage of TCR variable regions differ in responders vs. non-responders to treatment?	 How does TCR diversity change before and after treatment? Which TCR variable regions are being used and are any changes occurring? How does TCR diversity change before and after treatment with cell therapy? How does usage of TCR populations change after vaccination or disease recovery? 	 Which samples contain TCR populations desirable as a starting point for cell therapy?

Panel Content

There are four T cell Receptor Loci in Humans: Alpha, Beta, Gamma and Delta. The TCR Diversity Panel covers all functional variable and constant segments within each locus. Additionally, the panel covers key T cell markers to determine phenotype and activation status as well as a key marker of NK cells.

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	TRGC1, TRGC2	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)				

ROSALIND® Platform

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 www.rosalind.bio/nanostring

TCR Diversity Report

The TCR Diversity report is available for purchase separately from NanoString for use 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 and shown relative to designated grouping variables.

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.

There are three tabs within the report:

Summary	Heatmap	TCR Score
Plots for number of probes detected above background, broken down by variable regions and grouping variables selected for analysis. An explanation of the report and relevant assay QC information displayed in a table.	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.







Ordering Information

Product	Product Description	Quantity	Catalog Number
nCounter Human TCR Diversity Panel	Includes 129 genes; 10 internal reference genes for data normalization	12 Reactions	XT-CSO-HTCR 1-12
nCounter Human TCR Diversity Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the 119 unique probe targets in the panel.	12 Reactions	PSTD-TCRDIV-12
TCR Diversity Data Analysis Report	ROSALIND data analysis report for the nCounter TCR Diversity Panel. Minimum purchase of 12 samples.	1 Sample	ROSA-TCRDIV-1
nCounter Analysis System Master Kit Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing on the nCounter Analysis System	12 Reactions	NAA-AKIT-012
nCounter SPRINT Cartridge 1 Cartridge, 12 Ianes	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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Gene Expression Panels arrive ready-to-use and generally ship within 24 hours following purchase.

Selected Panel References

- 1. Jost L. Entropy and diversity. Oikos. 2006;113(2): 363-375.
- 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;18, 4733–4742.

To view the annotated gene lists for the TCR Diversity Panel visit nanostring.com/TCR-diversity

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