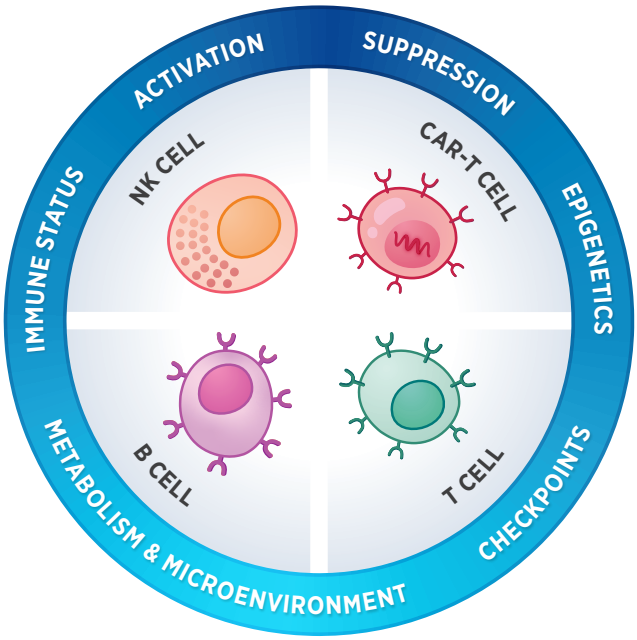
nCounter® Immune Exhaustion Panel

Immuno-oncology • CAR-T Cell Therapy • Infectious Disease • Drug Development

Uncover the mechanisms behind T cell, B cell, and NK cell exhaustion in diverse contexts, including cancer and infectious disease, with a 785 gene panel that gets you results in less than 24 hours and is compatible with a broad range of sample types. Characterize immune status, develop signatures for assessing the exhausted state, and identify novel therapeutic targets to prevent or reverse exhaustion.



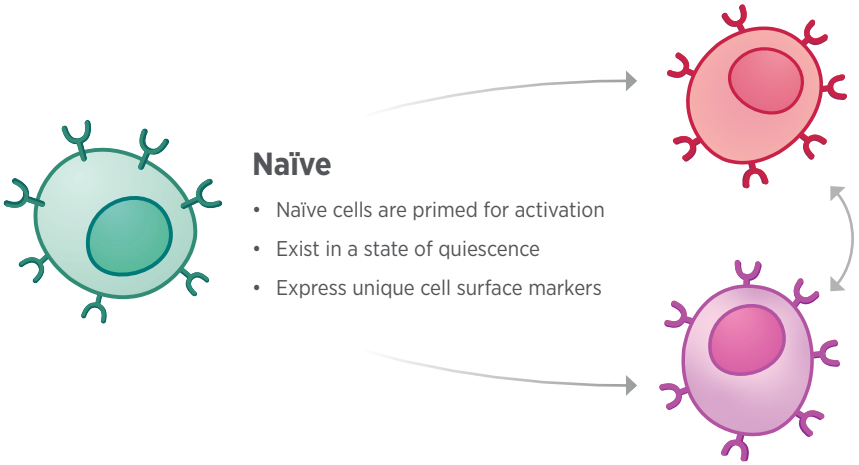
Product Highlights

- Directly profile 785 human and mouse genes across 47 pathways involved in immune exhaustion:
  - Immune Activation
  - Immune Suppression
  - Immune Status
  - Immune Checkpoints
  - Epigenetics
  - Metabolism & Microenvironment
- Understand the mechanisms of exhaustion in T cells, B cells, NK cells, CAR-T cells and other adoptive immune cells
- Discover novel therapeutic targets for preventing or reversing immune exhaustion
- Determine the extent of a peripherally suppressed, adaptive immune response to cancer with the 18-gene Tumor Inflammation Signature (TIS)
- Quantify the presence and relative abundance of 14 different immune cell types

Feature	Specifications
Number of Targets	785 (Human and Mouse), including 12 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 and Primer Pools (sold separately)
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

Characterizing Immune Status

Immune exhaustion results from a complex interplay between a cell and its environment. While checkpoint molecules and particular receptor signaling pathways within T cells, B cells, and NK cells are strong signals regulating exhaustion, senescence, anergy, and tolerance, these states are also influenced by cytokines and signaling from other cell types. Understanding immune exhaustion in the context of the overall immune cell status is an important assessment to make in order to determine the state of dysfunction, impact of therapeutics and the ability to intervene and reverse exhaustion.



Naïve

- Naïve cells are primed for activation
- Exist in a state of quiescence
- Express unique cell surface markers

Memory

- Exist in a quiescent state, not dividing or performing effector functions
- Capable of being re-activated and re-entering the cell cycle

Effector

- Effector cells perform their functions and actively divide

States of Effector Dysfunction

Exhaustion is not the only context where an immune cell may lack effector function. As such, understanding the similarities and distinctions between states of dysfunction is key to understanding immune exhaustion.

Anergic	Exhausted	Senescent
• Suboptimal stimulation • Low proliferative capacity • None or low effector function	• Persistent overstimulation • Low proliferative capacity • Low to moderate effector function • High expression of multiple inhibitory factors • Some exhausted cell subsets are reversible	• Repetitive Stimulation • Low proliferative capacity • Low expression of inhibitory receptors • High effector function • Irreversible

Pathway Annotations to Functional Themes

Immune Activation			Immune Suppression	Epigenetics	Immune Status	Immune Checkpoints	Metabolism & Microenvironment
Antigen Presentation	IL-1 Signaling	NK Receptors	IL-10 Signaling	Epigenetic Modification	Anergy	CTLA4 Signaling	Fatty Acid Metabolism
AP-1 Signaling	IL-2 Signaling	Other Interleukin Signaling	Myeloid Immune Evasion		B Cell Exhaustion	PD1 Signaling	Glutamine Metabolism
Apoptosis	IL-6 Signaling	PI3K-AKT Pathway	Notch Signaling		Naïve	T Cell Checkpoint Signaling	Glycolysis and Glucose Import
B Cell Memory	IL-7 Signaling	T Cell Memory	RAR Signaling		NK Exhaustion		Hypoxia Response
BCR Signaling	JAK/STAT Signaling	TCR Signaling	TGF-beta Signaling		Senescence & Quiescence		PPAR Signaling
Cell Cycle	MAPK Signaling	TLR Signaling			T Cell Exhaustion		
Chemokine Signaling	mTOR Signaling	TNF Signaling					
Cytotoxicity	NF-kB Signaling	Type I Interferon					
DAP12 Signaling	NK Activity	Type II Interferon					

Immune Checkpoint Coverage

The Immune Exhaustion Panel provides comprehensive coverage of the most relevant immune checkpoints that can potentially be used to modulate the dynamics of the immune response.

CD28	ICOSLG	LAG3	TNFSF18	ICOS
CD40	PDCD1	HAVCR2	VSIR	CD70
CD80	TNFSF4	TNFSF9	CD27	CD276
CD86	CD274	TNFRSF9	CD40LG	ADORA2A
CTLA4	PDCD1LG2	TNFRSF18	TNFRSF4	TIGIT

Viral Identification

Chronic infections caused by viruses and other pathogens can induce immune exhaustion. The Human Immune Exhaustion Panel includes probes for Epstein-Barr virus (EBV) and Cytomegalovirus (CMV), and the Mouse Immune Exhaustion Panel includes probes for Lymphocytic Choriomeningitis (LCMV). The panel can be supplemented with up to 55 genes of your choice with a Panel Plus spike-in for studying exhaustion in the context of different types of infectious disease.

Tumor Inflammation Signature

The 18-gene Tumor Inflammation Signature (TIS) is included in the panel gene list and measures activity known to be associated with PD-1/PD-L1 inhibitors. Customers have the option to purchase a standalone TIS report with the Immune Exhaustion Panel.

- Includes four axes of biology that characterize a peripherally suppressed, adaptive immune response, including:
  - Antigen presenting cells
  - T cell/NK cell presence
  - IFNγ biology
  - T cell exhaustion
- Tissue-of-origin agnostic (Pan-Cancer)
- Potential surrogate for PD-L1 and mutational load in a research setting

Immune Cell Profiling Feature

Genes included in the Immune Exhaustion Panel provide unique cell profiling data to measure the relative abundance of 14 different immune cell types. The table below summarizes the genes included in each cell type signature, as qualified through biostatistical approaches and selected literature in the field of immunology.

Cell Type	Genes
B cells	BLK, CD19, FAM30A, FCRL2, MS4A1, PNOC, SPIB, TCL1A, TNFRSF17
CD45	PTPRC
CD8 T cells	CD8A, CD8B
Cytotoxic cells	CTSW, GNLY, GZMA, GZMB, GZMH, KLRB1, KLRD1, KLRK1, NKG7, PRF1
Dendritic cells	CCL13, CD209, HSD11B1
Exhausted CD8	CD244, EOMES, LAG3, PTGER4
Macrophages	CD163, CD68, CD84, MS4A4A
Mast cells	CPA3, HDC, MS4A2, TPSAB1/B2
NK CD56dim cells	IL21R, KIR2DL3/4, KIR3DL1/2
NK cells	NCR1, XCL1/2
Neutrophils	CEACAM3, CSF3R, FCAR, FCGR3A/B, FPR1, S100A12, SIGLEC5
T cells	CD3D, CD3E, CD3G, CD6, SH2D1A, TRAT1
Th1 cells	TBX21
Treg	FOXP3

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 Immune Exhaustion Panel are organized and linked to various advanced analysis modules to allow for efficient analysis of 47 annotated pathways.

Analysis Modules available for Immune Exhaustion:

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

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

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 Immune Exhaustion Panel	Gene Expression CodeSet profiling (785 genes) 773 human immune response genes + 12 internal reference controls. No Master Kit.	12 Reactions	XT-H-EXHAUST-12
nCounter Human Immune Exhaustion Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the 785 unique probe targets in the panel.	12 Reactions	PSTD-H-EXHAUST-12
nCounter Human Immune Exhaustion Panel Primer Pool	H Exhaustion Primers (for use with Low RNA Input Kit)	12 Reactions	LOW-H-EXHAUST-12
nCounter Mouse Immune Exhaustion Panel	Gene Expression CodeSet profiling (785 genes) 773 mouse immune response genes + 12 internal reference controls. No Master Kit.	12 Reactions	XT-M-EXHAUST-12
nCounter Mouse Immune Exhaustion Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the 785 unique probe targets in the panel.	12 Reactions	PSTD-M-EXHAUST-12
nCounter Mouse Immune Exhaustion Panel Primer Pool	M Exhaustion Primers (for use with Low RNA Input Kit)	12 Reactions	LOW-M-EXHAUST-12
Low RNA Input Kit	Kit for use with all Low RNA Input Primer Pools	48 Reactions	LOW-RNA-48
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 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. Baitsch L et al. Exhaustion of tumor-specific CD8+ T cells in metastases from melanoma patients. J. Clin. Invest. 2011;121(6):2350-60.
3. Beldi-Ferchiou A et al. PD-1 mediates functional exhaustion of activated NK cells in patients with Kaposi sarcoma. Oncotarget. 2016;7(45):72961-72977.
4. Jelicic K et al. The HIV-1 envelope protein gp120 impairs B cell proliferation by inducing TGF-β1 production and FcRL4 expression. *Nat. Immunol.* 2013;14(12): 1256–1265.
5. Jiang Y et al. T-cell exhaustion in the tumor microenvironment. *Cell Death & Disease* 2015;6(6):e1792.
6. Khan O et al. TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. *Nature* 2019;571(7764):211-218.
7. Moir S & Fauci AS. B-cell exhaustion in HIV infection: the role of immune activation. *Current Opinion in HIV and AIDS* 2014;9(5):472-7.
8. Wherry EJ & Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015;15(8):486-99.

To view the annotated gene lists for the Immune Exhaustion Panel, visit nanostring.com/immune-exhaustion

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