nCounter® ADC Development Panel

Gene Expression Panel

Target & Drug Screening • Mechanism of Action Studies • Traditional & Combination Therapies

The novel nCounter® ADC Development Panel enables researchers to answer complex questions critical for the success of Antibody Drug Conjugates throughout discovery, pre-clinical and clinical development. Biological function can now be assessed using quantitative molecular characterization spanning 6 stages in the lifecycle of the ADC. The comprehensive gene content covers the biology of mechanisms of resistance, immunogenic cell death, aspects of the immune response, as well as traditional and emerging MOAs. Markers covering current and developing targets for ADCs are also included. The success of both traditional chemotherapy and immunotherapy as part of a combination treatment can be evaluated, and the panel can be customized with tumor-specific or ADC-specific targets of interest.

Product Highlights

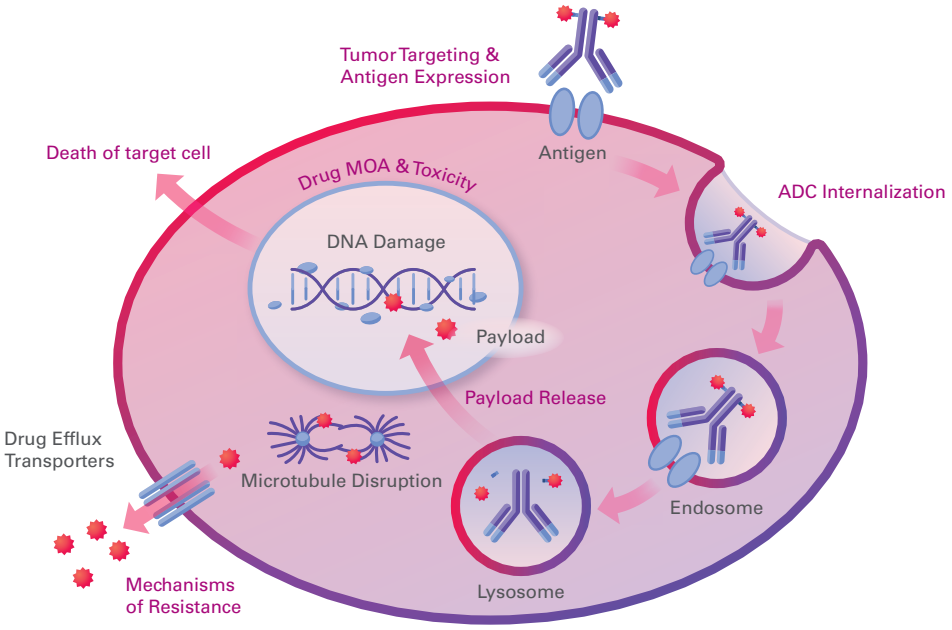
- Directly profile 770 human or mouse genes that address essential biological questions relevant to each stage of ADC development
  - Tumor Targeting & Antigen Expression
  - ADC Internalization
  - Payload Release
  - Drug MOA
  - Target Cell Death
  - Mechanisms of Resistance
- Address biological function with deep molecular characterization, expanding insights gained from traditional endpoint assays
- Compatible with a variety of sample types, including treated cell lines (both in vivo and in vitro), tumor biopsies, xenografts, and mouse cells
- Quantify the presence and relative abundance of 14 different immune cell types
- Generate data in 24 hours with less than 30 minutes hands on time and simple data analysis

Feature	Specifications
Number of Targets	770 (Human and Mouse), including 20 internal reference genes designed for compatibility across the PanCancer panels
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)	Tumor biopsies, xenografts, cultured cells/cell lysates, sorted cells, FFPE-derived RNA, total RNA, fragmented RNA
Customizable	Add up to 55 unique genes with Panel Plus
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software (RUO), Advanced Analysis for cell profiling, the ROSALIND® platform, TIS Data Analysis Report (fee-based analysis service)

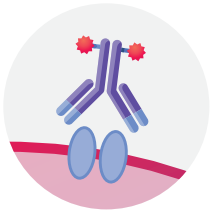
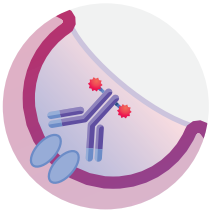
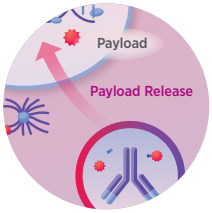
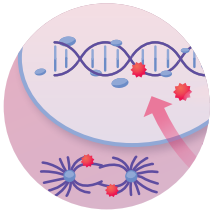
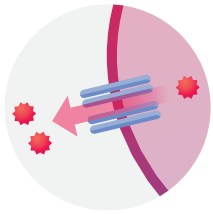
The ADC Development Process

The ADC Development Panel can be used throughout the ADC development process to characterize all the essential stages of ADC function.

The ADC Mechanism of Action with Key Panel Focus Areas



Examples of Key Biological Questions Addressed by the ADC Panel

Tumor Targeting & Antigen Expression	ADC Internalization	Payload Release	Drug MOA & Toxicity	Death of Target Cells	Mechanisms of Resistance
					
What antigens are on the target cell? What is the degree of expression? How specific is the antigen to cancer cells relative to normal cells?	Did the cell internalize the ADC?	Did the linker separate? What possible enzymes could catalyze linker cleavage?	Are there aspects of tumor biology that might prevent response? What is the state of the immune system and is it primed to respond to the targeted tumor cells?	What is the state of the various pathways that lead to cell death via ADCs?	Are there pumps that could prevent the payload from remaining in the target cell?

Functional Annotations

The ADC Development Panel measures 6 distinct stages of ADC delivery and response in a single gene expression panel, gauging the success of both traditional chemotherapy and combination immunotherapy. Pathway coverage is outlined in the table below.

The 6 Stages of ADC Delivery and Response

Tumor Targeting & Antigen Expression	ADC Internalization	Payload Release	Drug MOA & Toxicity	Death of Target Cells	Mechanisms of Resistance
• Tumor Antigens	• Endocytosis • Sialic Acid Metabolism	• Enzymes • Lysosome	• Cell Cycle • DNA Damage Pathways • Innate Immune Pathways • Cellular Stress Pathways • DNA Replication • RNA Polymerase • Spliceosome • Tubulin Expression	• Apoptosis • Antibody Dependent Cellular Cytotoxicity (ADCC) • Complement Dependent Cytotoxicity (CDC) • Antibody Dependent Cellular Phagocytosis (ADCP) • Immunogenic Cell Death • Necroptosis	• Drug Efflux Pumps • Tumor Stroma • Vasculature & Permeability

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 ADC Development Panel.

- Includes four axes of biology that characterize a peripherally suppressed, adaptive immune response, including:
  - Antigen presenting cells
  - T cell/NK cell presence
  - Interferon gamma 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 ADC Development Panel provide unique cell profiling data to measure the relative abundance of 14 different immune cell types. These markers that identify specific immune cell types can efficiently define both the immunological activity of the samples as well as identify changes in immune cell populations in response to external stimuli from payload release. The table summarizes the genes included in each cell type signature, as qualified through biostatistical approaches and selected literature in the field of immunology.

Immune Cell Profiling Feature

Cell Type	Associated Human 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
DC	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, 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

Contamination Detection

Mycoplasma is a common contaminant in cultured cells. Mycoplasma compete with cells for nutrients and can have a profound impact on global gene expression levels within the cells. The ADC Development Panel contains a probe to detect mycoplasma, allowing for quick and easy detection of culture contamination when using cell-based assays to understand ADC activity. The panel can also be customized by adding up to 55 genes of your choice with a Panel Plus spike-in for studying additional sources of potential contamination.

Customization with Panel Plus

Customize your research project by adding up to 55 user-defined genes of interest with nCounter Panel Plus. Panel Plus capacity enables researchers to address content specific to the cancer type they are studying or specific ADC targets of interest.

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 ADC Development Panel are annotated to allow for efficient analysis of relevant pathways.

Analysis Modules available for ADC Development:

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

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 free of charge at www.rosalind.bio/nanostring

ROSALIND

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 ADC Development Panel	770 genes, including 20 internal reference genes for data normalization	12 Reactions	XT-HSADC-12
nCounter® Mouse ADC Development Panel	770 genes, including 20 internal reference genes for data normalization	12 Reactions	XT-MSADC-12
nCounter® ADC Development Primer Pool	Low input protocol and primer designs available	N/A	Ask Your Sales Rep
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

Selected Panel References

1. Nakanishi, T. & Tamai, I. Solute Carrier Transporters as Targets for Drug Delivery and Pharmacological Intervention for Chemotherapy. Journal of Pharmaceutical Sciences 100, 3731–3750 (2011).
2. Azzi, S., Hebda, J. K. & Gavard, J. Vascular Permeability and Drug Delivery in Cancers. Front. Oncol. 3, (2013).
3. Moek, K. L., de Groot, D. J. A., de Vries, E. G. E. & Fehrmann, R. S. N. The antibody–drug conjugate target landscape across a broad range of tumour types. Annals of Oncology 28, 3083–3091 (2017).
4. Staudacher, A. H. & Brown, M. P. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required? Br J Cancer 117, 1736–1742 (2017).
5. Zhou, J. et al. Immunogenic cell death in cancer therapy: Present and emerging inducers. J Cell Mol Med 23, 4854–4865 (2019).
6. Coats, S. et al. Antibody–Drug Conjugates: Future Directions in Clinical and Translational Strategies to Improve the Therapeutic Index. Clin Cancer Res 25, 5441–5448 (2019).
7. Yaghoubi, S. et al. Potential drugs used in the antibody–drug conjugate (ADC) architecture for cancer therapy. J Cell Physiol 235, 31–64 (2020).
8. Ponziani, S. et al. Antibody-Drug Conjugates: The New Frontier of Chemotherapy. IJMS 21, 5510 (2020).
9. Hafeez, U., Parakh, S., Gan, H. K. & Scott, A. M. Antibody–Drug Conjugates for Cancer Therapy. Molecules 25, 4764 (2020).
10. Criscitiello, C., Morganti, S. & Curigliano, G. Antibody–drug conjugates in solid tumors: a look into novel targets. J Hematol Oncol 14, 20 (2021).

For more information, please visit nanostring.com/ADCdevelopment

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