

nCounter® PanCancer IO 360™ Panel

Gene Expression Panel

Signature Development • Evasion Mechanisms • Tumor Biology

The nCounter PanCancer IO 360 panel and data analysis service provides a unique 360 degree view of gene expression for the tumor, microenvironment and immune response. Now researchers can more quickly evaluate immune evasion by incorporating 48 potentially predictive biological signatures including the Tumor Inflammation Signature.¹



Product Highlights

- Expertly curated, comprehensive content includes 770 human or mouse genes across 16 key immuno-oncology pathways and processes
- 48 signatures including the Tumor Inflammation Signature, 14 signatures measuring immune cell populations, and 34 novel signatures measuring important tumor and immune activities
- Streamlined analysis with access to validated and novel research signatures including the Tumor Inflammation Signature along with 11 key individual immune response genes
- Easy to use nCounter system provides data in 24 hours with less than 30 minutes hands on time

| Feature | Specifications |
|---|--|
| Number of Targets | 770 (Human), 770 (Mouse) including internal reference genes |
| Sample Input - Standard (No amplification required) | 50 ng - 300 ng |
| Panel Standard | Synthetic oligonucleotide pool corresponding to all panel gene targets used for normalization. |
| Low Input Material | As little as 1 ng with nCounter RNA Low Input Kit; low input protocol and primer designs available |
| Sample Type(s) | FFPE-derived RNA, total RNA, fragmented RNA, cell lysates and PBMCs |
| Customizable | Add up to 55 unique genes with Panel Plus |
| Time to Results | Approximately 24 hours |
| Data Analysis | nSolver™ Analysis software, IO 360 Data Analysis Report, and the ROSALIND® Platform |

Comprehensive Content Across Tumor-Microenvironment-Immune Response

PanCancer IO 360 Pathways and Processes

| Tumor Foreignness | | | Tumor Microenvironment | | | Immune Activity | | |
|---------------------------------|-------------|-------------|-------------------------------------|-------------|-------------|--|-------------|-------------|
| Category | Human Genes | Mouse Genes | Category | Human Genes | Mouse Genes | Category | Human Genes | Mouse Genes |
| Release of Cancer Cell Antigens | 74 | 69 | Angiogenesis | 40 | 41 | T Cell Priming and Activation | 151 | 152 |
| Cell Cycling and Proliferation | 54 | 53 | Extracellular Matrix Remodelling | 43 | 41 | Killing of Cancer Cells | 177 | 173 |
| Tumor Intrinsic Factors | 156 | 149 | Collagens | 6 | 6 | Recognition of Cancer Cells by T Cells | 103 | 104 |
| Common Signaling Pathways | 172 | 162 | Metastasis | 20 | 20 | NK Cell Activity | 28 | 27 |
| Cancer Antigen Presentation | 95 | 95 | Immunometabolism | 99 | 101 | Myeloid Cell Activity | 262 | 258 |
| | | | Immune Cells Localization to Tumors | 293 | 291 | | | |

PanCancer IO 360 Biological Signatures

Content included in the IO 360 panel allows for calculation of 48 gene signatures measuring biological variables crucial to the tumor-immune interaction. Both analytically validated and research signatures are enriched with potentially predictive genes encompassing seven different biological functions measuring antigen availability, structural barriers to immune infiltration, inhibitory signaling, inhibitory metabolism, pro-immune signaling, killing of tumor cells, tumor receptiveness to immune signaling, tumor proliferation and apoptosis.

- 48 signatures allow for a multi-faceted view of the tumor immune interplay including the Tumor Inflammation Signature
- Novel research signatures trained through a combination of domain expertise, literature review and TCGA analysis along with ongoing refinement through our data analysis service
- Confirmation of signature accuracy and interpretation involved stringent testing with only signatures displaying strong evidence for measuring biological activity included

| Tumor Immunogenicity | Tumor Sensitivity to Immune Attack | Inhibitory Immune Mechanisms | Stromal Factors | Inhibitory Metabolism | Anti-Tumor Immune Activity | Inhibitory Immune Signaling | Immune Cell Population Abundance | |
|--|---------------------------------------|------------------------------|--------------------------|-----------------------|------------------------------------|--|----------------------------------|-----------------------------------|
| Antigen Processing Machinery | Apoptosis | IDO1 Gene Expression | Endothelial Cells | Glycolysis | Tumor Inflammation Signature (TIS) | CTLA4 Gene Expression | B Cells | NK CD56dim Cells |
| Antigen Presenting Machinery Expression Loss | Tumor Proliferation | PD-L1 Gene Expression | Stromal Tissue Abundance | Hypoxia | Cytotoxicity | IL10 Gene Expression | CD45+ Cells | Natural Killer Cell Abundance |
| Immunoproteasome | JAK-STAT Pathway Gene Expression Loss | B7-H3 Gene Expression | | | Interferon Gamma Signaling | Inflammatory Chemokines | CD8 T Cell | T Cells Abundance |
| MAGE Genes Expression | | TGF-Beta Gene Expression | | | Interferon Signaling Response | Myeloid-Derived Inflammatory Signaling | Cytotoxic Cells | TH1 Cell (TBX21/ Tbet) Expression |
| Loss of Mismatch Repair Gene Expression | | | | | Lymphoid Compartment Activity | PD-1 Gene Expression | Dendritic Cells | Treg (FOXP3 Expression) |
| Hypermutation | | | | | MHC Class II Antigen Presentation | PD-L2 Gene Expression | Exhausted CD8 Cell | |
| MSI Predictor | | | | | Myeloid Compartment Activity | TIGIT Gene Expression | Macrophage | |
| | | | | | | ARG1 Gene Expression | Mast Cells | |
| | | | | | | NOS2 Gene Expression | Neutrophils | |

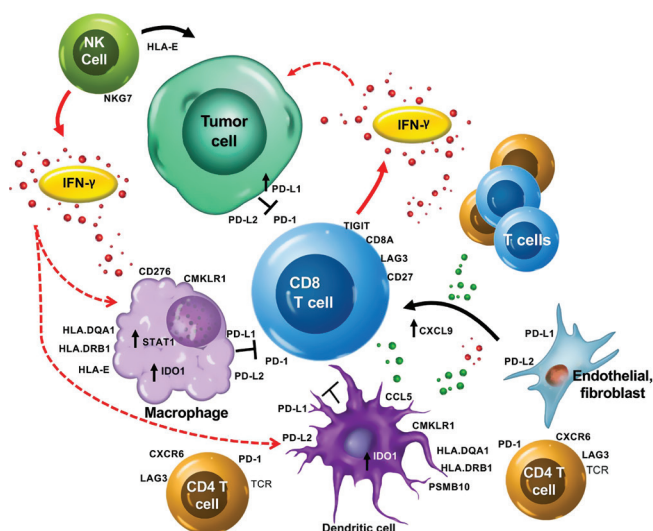
Included within the IO 360 panel is the Tumor Inflammation Signature. This 18-gene signature measures activity known to be associated with response to PD-1/PD-L1 inhibitors pathway blockade.

- ## PanCancer IO 360 Data Analysis Services

A detailed signature analysis report for the PanCancer IO 360 panel is available to enrich interpretation of the profiling. Interactive reports and individual consultation provide information on all 48 signatures and 770 genes included in the panel.

- Customized, interactive reports prepared by NanoString's expert Scientists
- Tumor Inflammation Score provided for each sample to determine "hot" and "cold" tumors
- All your data undergoes QC and normalization (up-front)
- Includes a one hour report out and consultation with an expert analyst
- Up to 5 additional customer-defined signatures can be included in the analysis based on IO 360 or Panel Plus genes

An interactive 360 Signature Analysis Report and consultation services for the PanCancer IO 360 panel further enrich the interpretation of the profiling data. This service available through our Data Analysis Services Program. Please inquire for pricing.



TIS = 9.06

| Category | Term | Value (approx.) |
|----------------------------------|---------------------|-----------------|
| Anti-Tumor Immune Activity | TIS | 9.06 |
| | IFN Gamma | 1.5 |
| | IFN Downstream | 1.5 |
| | Cytotoxicity | 1.5 |
| | Proliferation | 1.5 |
| | Apoptosis | 1.5 |
| | MAGEs | 1.5 |
| | APM | 1.5 |
| | Stroma | 1.5 |
| | Endothelial Cells | 1.5 |
| Immune Cell Population Abundance | TGF-Beta | 1.5 |
| | PD-L1 | 1.5 |
| | IDO1 | 1.5 |
| | B7-H3 | 1.5 |
| | Glycolytic Activity | 1.5 |
| | IL-10 | 1.5 |
| | CTLA4 | 1.5 |
| | Treg | 1.5 |
| | Th1 Cells | 1.5 |
| | T Cells | 1.5 |
| Inhibitory Immune Signaling | NK Cells | 1.5 |
| | NK CD56dim | 1.5 |
| | Neutrophils | 1.5 |
| | Mast Cells | 1.5 |
| | Macrophages | 1.5 |
| | CD8 | 1.5 |
| | DC | 1.5 |
| | CD3 | 1.5 |
| | CD4 | 1.5 |
| | CD8 T Cells | 1.5 |
| Inhibitory Metabolism | Exhausted | 1.5 |
| | Cytotoxic Cells | 1.5 |
| | Myeloid | 1.5 |
| | MHC2 | 1.5 |
| | Lymphoid | 1.5 |
| | IFN | 1.5 |
| | IFN Gamma | 1.5 |
| | Cytotoxicity | 1.5 |
| | Proliferation | 1.5 |
| | Apoptosis | 1.5 |

Signature scores are shown for the selected sample, with TIS in the center. Scores range from approximately 0-10; for most scores, a value of 5 is average. Each unit increase in score corresponds to a doubling of the biological process it measures. Color denotes each signature's biological function.

| Product | Product Description | Quantity | Catalog Number |
|---|---|---|------------------------|
| nCounter Human PanCancer IO 360 Panel | Gene Expression CodeSet profiling (770 genes) 750 cancer-related human genes + 20 internal reference controls. No Master Kit. | 12 Reactions | XT-CSO-HIO360-12 |
| nCounter Human PanCancer IO 360 Panel Standard | Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the unique probe targets in the panel. | 12 Reactions | PSTD-HIO360-12 |
| nCounter Mouse PanCancer IO 360 Panel | Gene Expression CodeSet profiling (770 genes) 750 cancer-related mouse genes + 20 internal reference controls. No Master Kit. | 12 Reactions | XT-CSO-MIO360-12 |
| nCounter Mouse PanCancer IO 360 Panel Standard | Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the unique probe targets in the panel. | 12 Reactions | PSTD-MIO360-12 |
| nCounter Master Kit | 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 |
| Low Input RNA Reagent Kit | 48rxn kit for profiling from low sample input amounts | 48 Reactions | LOW-RNA-48 |
| Human PanCancer IO 360 Primer Pool | Low input protocol and primer designs available. | N/A | Contact Your Sales Rep |
| Mouse PanCancer IO 360 Primer Pool | Low input protocol and primer designs available. | N/A | Contact Your Sales Rep |
| IO 360 Data Analysis Report | Data analysis report for PanCancer IO 360 Panel | Report purchased in 12 sample increments | Contact your local rep |
| IO 360 Data Analysis Service for PanCancer Immune Profiling Panel | Data analysis service for PanCancer Immune Profiling Panel | Single assay or large dataset project pricing available | DAS-IOPCIP360-1 |

Selected Panel References

1. Ayers, Mark, et al. "IFN- γ -related mRNA profile predicts clinical response to PD-1 blockade." *The Journal of Clinical Investigation* 127.8 (2017).
2. Danaher, Patrick, et al. "Gene expression markers of Tumor Infiltrating Leukocytes." *Journal for immunotherapy of cancer* 5.1 (2017): 18.
3. Satoh, Jun-ichi, and Hiroko Tabunoki. "A comprehensive profile of ChIP-Seq-based STAT1 target genes suggests the complexity of STAT1-mediated gene regulatory mechanisms." *Gene regulation and systems biology* 7 (2013): 41.
4. Becht, Etienne, et al. "Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression." *Genome biology* 17.1 (2016): 218.
5. Spranger, Stefani, Riyue Bao, and Thomas F. Gajewski. "Melanoma-intrinsic [beta]-catenin signalling prevents anti-tumour immunity." *Nature* 523.7559 (2015): 231.
6. Harris, B. H. L., et al. "Gene expression signatures as biomarkers of tumour hypoxia." *Clinical Oncology* 27.10 (2015): 547-560.
7. Manson, G., et al. "Biomarkers associated with checkpoint inhibitors." *Annals of Oncology* 27.7 (2016): 1199-1206.
8. Blank, Christian U., et al. "The 'cancer immunogram'." *Science* 352.6286 (2016): 658-660.

For more information visit nanosttring.com/IO360Panel

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