# nanoString

## GeoMx® Protein Assays for Immuno-Oncology

Designed with a modular design to provide flexible and comprehensive coverage of the tumor and tumor microenvironment, profile up to 96 curated protein targets with spatial resolution from a single tissue section using the GeoMx Digital Spatial Profiler (DSP).

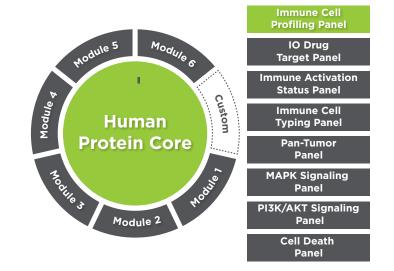


#### **Product Highlights**

- Curated content designed for immuno-oncology and immunology research
- Modular design allows selection of up to six 10-plex modules to add to the Immune Cell Profiling Core
- Pre-validated in multiplex format for use in human and mouse FFPE or fresh frozen tissue
- Customizable with up to 10 additional targets of interest
- For use with nCounter readout and compatible with DSP Data Center Software

#### GeoMx Protein Assay Design

Designed to profile up to 96 targets simultaneously with spatial resolution. The Immune Cell Profiling Core contains 18 targets for broad cell profiling and includes necessary controls for all GeoMx DSP experiments. Up to 6 modules plus 10 custom targets can be added to select the content most relevant to your research. All cores and modules contain validated antibodies conjugated to unique DNA indexing-oligonucleotides via a UV-photocleavable linker. After region of interest (ROI) selection on GeoMx DSP and UV cleavage of the oligonucleotides, each DNA oligonucleotide is recognized by a unique reporter probe that contains a fluorescent barcode. Reporter probes are imaged and counted by the nCounter® Analysis System to provide a direct, digital readout of spatially resolved protein expression.



#### **Curated Content for Immuno-Oncology**

- Immune Cell Profiling Core: Includes key immuno-oncology targets and markers of immune cell types, including T cells, B cells, macrophages, NK cells, epithelia, and stroma. It also contains the controls needed to run any GeoMx DSP experiment.
- **IO Drug Target Module:** Includes drug targets in development within the immuno-oncology space, including checkpoint molecules and metabolic mediators of immune function.
- Immune Activation Status Module: Includes additional checkpoint molecules that modulate T cell activation.
- Immune Cell Typing Module: Includes an expanded set of cell type markers to more deeply profile immune cell types covered in the Immune Cell Profiling Core and measure additional immune cell types, including T cell subsets.
- **Pan-Tumor Module:** Includes an expanded set of markers for detecting specific tumor types, including ER+/HER2+ breast tumors, hematopoietic malignancies, and melanoma.
- Cell Death Module: Includes protein mediators of immunogenic and programmed cell death.
- **PI3K/AKT Signaling Module:** Includes key proteins involved in PI3K-AKT signal transduction, and phosphorylated protein products that measure pathway activation.
- **MAPK Signaling Module:** Includes key proteins involved in MAPK signal transduction, and phosphorylated protein products that measure pathway activation.

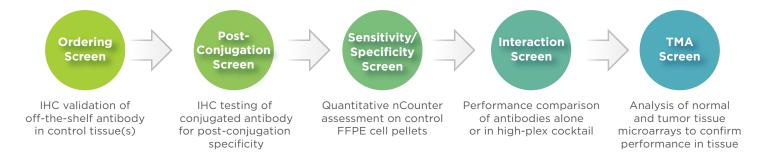
Accompanying Morphology Marker Kits are available for tissue visualization and ROI selection.

Immune Cell Profiling Panel Human Protein Core					IO Drug Target Panel Human Protein Module					
Beta-2-microglobu	lin CD3	C	D56 CTLA4			GZMB	PD	)-1	4-1BB	LAG3
CD11c	CD4	С	D68	Pan-cytokera	atin	HLA-DR	PD	-L1	ARG1	OX40L
CD20	CD45	(	CD8	Fibronectin		Ki-67	SM	IA	B7-H3	STING
	Ms IgG2a					Histone H3	3 GI			TIM-3
	Ms IgG1				S6				ID01	VISTA
	Rb IgG					GAPDH				
Immune Activation Status PanelCell Death PanelHuman Protein ModuleHuman Protein Module				PI3K/AKT Signaling Panel Human Protein Module						
CD127	CD80	BAD	GZI	MA		Pan-AKT		Phospho-GSK3A (S21)/Phospho-GSK3B (		hospho-GSK3B (S9)
CD25	ICOS	BCL6	p5	53		MET		INPP4B		В
CD27	PD-L2	BCLXL	PA	RP	Pho	ospho-AKT1 (S47	73)	PLCG1		1
CD40		BIM	Cleaved C	Caspase 9	Pho	ospho-GSK3B (S	59)	Phospho-PRAS40 (T246)		40 (T246)
CD44		CD95/Fas	Neurofi	bromin	Phos	pho-Tuberin (T1	462)			
Immune Cell Typing Panel Pan-Tumor Panel Human Protein Module Human Protein Module				MAPK Signaling Panel Human Protein Module						
CD14	FAPalpha	BCL-2	NY	'-ESO-1	EGFR			Phospho-MEK1 (S217/S221)		
CD163	FOXP3	EpCAM		PR pan-RAS Phospho-p38 MAPK		K (T180/Y182)				
CD34		ER alpha	a F	PTEN		BRAF	I	Phospho-p44/42 MAPK ERK1/2 (T202/Y20		RK1/2 (T202/Y204)
CD45RO		HER2/ERB	B2 S	100B	Pho	spho-c-RAF (S3	38)	p44/42 MAPK ERK1/2		K ERK1/2
CD66b		MART1			Phosp	pho-JNK (T183/\	Y185)	Phospho-p90 RSK (T359/S36		(T359/S363)

Immune Cell Profiling Panel Mouse Protein Core			IO Drug Tai Mouse Prote		Immune Activation Status Panel Mouse Protein Module				
PD-1	CD11c	CD8a	PanCk	LAG3	B7-H3	CD127/IL7RA		CD44	
				OX40L	GITR	CD27		CD86	
MHC II	CD19	CTLA4	SMA	Tim-3		С	D40	ICOS	
CD11b	CD3e	Fibronectin		VISTA		CD40L			
Ki-67	CD4	GZMB		Immune Cell T Mouse Prote		PI3K/AKT Signaling Panel Mouse Protein Module			
F4/80	CD45	PD-L1		CD14	FOXP3	Phospho-AKT1 (S473)		PLCG1	
Rb	Rb IgG Histone H3		CD163	Ly6G/Ly6C	Phospho-S6 (S235/S236)		Phospho-PRAS40 (T246)		
Rt Ig	JG2a	S	6	CD31	BatF3	Phospho-AMPK-alpha (T172) Pan-A		Pan-AKT	
Rt Ig	IG2b	GAF	DH	CD34	CD28	Phospho-GSK3A (S21)/ MET Phospho-GSK3B (S9)		MET	
				umor Panel rotein Module			MAPK Signaling Panel Mouse Protein Module		
BAD		p53	IFNGR	GFP	В	RAF	p38 MAPK		
Cleaved casp	base 3	Perforin	Epcam	Pmel17	E	GFR Phospho-p4		/42 MAPK ERK1/2 (T202/Y204)	
BCLXL		PARP	S100B	Her2	M	MEK1		pan-RAS	
gamma-H2	2AX	BIM	ER	AhR	Phospho-JN	IK (T183/Y185)	(T183/Y185) p44/42 MAPK ERK1/2		
p21			AR		Phospho-ME	no-MEK1 (S217/S221) Phospho-ps		p90 RSK (T359/S363)	

#### Validated Assays Ready for Use

All GeoMx Protein Assays undergo extensive validation to ensure high quality GeoMx DSP data.



#### Spatial Protein Profiling with High Specificity

Protein detection shows high specificity pre- and post-oligonucleotide conjugation (FIGURE 1).

Additionally, spike-in of each module to the Immune Cell Profiling Core does not alter specificity of the antibodies, demonstrating robust multiplex performance (**FIGURE 2**).

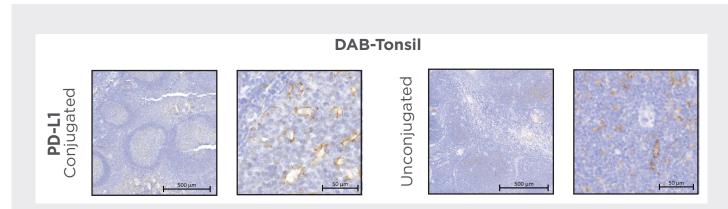


FIGURE 1: Example PD-L1 (DPROT\_00004.1) is tested for specific staining pre- and post-conjugation to a specific indexing-oligonucleotide to ensure conjugation does not alter specificity.

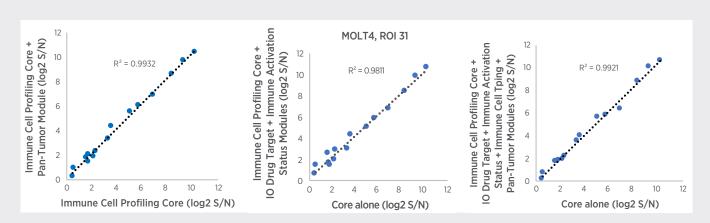


FIGURE 2: Example signal for the Immune Cell Profiling Core is compared to the Immune Cell Profiling Core plus individual Modules to ensure no antibody-antibody interference in MOLT4 cell lines. Single antibody interference testing is also performed prior to combined core and module testing.

#### **Reveal Tissue Heterogeneity**

Analysis of mixed tumor and tumor microenvironment ROI from non-small cell lung cancer (NSCLC) show distinct protein expression profiles in each segment respectively (**FIGURE 3**).

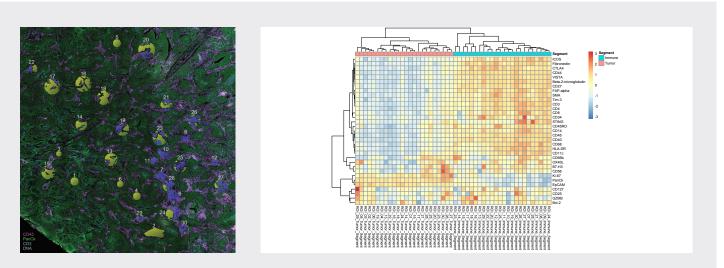


FIGURE 3: ROI were selected with mixed tumor and tumor microenvirontment (immune) segments in NSCLC FFPE tissue. ROI were segmented based on PanCK/CD45 morphology stain. Protein expression shows strong clustering by compartment.

#### GeoMx<sup>®</sup> Data Center

Unique GeoMx software combines whole tissue visualization at single cell resolution with advanced ROI selection to enable comprehensive spatial profiling of tissue sections. The fully integrated workflow tracks image data to corresponding profiling data, allowing users to easily go from data collection to data analysis and to interact with either dataset in real time.

The data analysis module assesses the quality of the raw data and provides a number of options to normalize data sets. Moreover, a variety of data visualization formats are enabled to export publication-quality figures. Visualization plots include: heatmap, cluster, bar graph, box plot, scatter plot, line/trend plot, strip plot, volcano plot, and PCA.

## **Ordering Information**

GeoMx Protein Assays					
Product	Product Description	Quantity	Catalog Number		
GeoMx Immune Cell Profiling Panel Human Protein Core for nCounter	Protein core including 18 targets for human immune cell profiling plus positive and negative controls. Includes AbMix and Probe R1 for 48 AOI per slide. Can be run as a standalone panel.	12 slides	GMX-PROCO- NCT-HICP-12		
GeoMx IO Drug Target Panel Human Protein Module for nCounter	Protein module including 10 targets for human immuno-oncology drug targets. Includes AbMix and Probe R2 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-HIODT-12		
GeoMx Immune Activation Status Panel <i>Human Protein Module for nCounter</i>	Protein module including 8 targets for human immune activation status. Includes AbMix and Probe R3 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-HIAS-12		
GeoMx Immune Cell Typing Panel Human Protein Module for nCounter	Protein module including 7 targets for human immune cell typing. Includes AbMix and Probe R4 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-HICT-12		
GeoMx Pan-Tumor Panel Human Protein Module for nCounter	Protein module including 9 targets for human pan-tumor analysis. Includes AbMix and Probe R5 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-HPT-12		
GeoMx Cell Death Panel Human Protein Module for nCounter	Protein module including 9 targets for human cell death. Includes AbMix and Probe R6 for 48 AOI per slide. Must be run with a protein core. Currently available for pre-order. Shipment schedule will be confirmed at a later date.	12 slides	GMX-PROMOD- NCT-HCD-12		
GeoMx PI3K/AKT Signaling Panel Human Protein Module for nCounter	Protein module including 9 targets for human PI3K/AKT signaling. Includes AbMix and Probe R7 for 48 AOI per slide. Must be run with a protein core. Currently available for pre-order. Shipment schedule will be confirmed at a later date.	12 slides	GMX-PROMOD- NCT-HPI3K-12		
GeoMx MAPK Signaling Panel Human Protein Module for nCounter	Protein module including 10 targets for human MAPK signaling. Includes AbMix and Probe R4 for 48 AOI per slide. Must be run with a protein core. Currently available for pre-order. Shipment schedule will be confirmed at a later date.	12 slides	GMX-PROMOD- NCT-HMAPK-12		
GeoMx Immune Cell Profiling Panel Mouse Protein Core for nCounter	Protein core including 17 targets for mouse immune cell profiling plus positive and negative controls. Includes AbMix and Probe R1 for 48 AOI per slide. Can be run as a stand alone panel.	12 slides	GMX-PROCO- NCT-MICP-12		
GeoMx IO Drug Target Panel Mouse Protein Module for nCounter	Protein module including 6 targets for mouse immuno-oncology drug targets. Includes AbMix and Probe R2 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-MIODT-12		
GeoMx Immune Activation Status Panel Mouse Protein Module for nCounter	Protein module including 7 targets for mouse immune activation status. Includes AbMix and Probe R3 for 48 AOI per slide. Must be run with a protein core. Currently available for pre-order. Shipment schedule will be confirmed at a later date.	12 slides	GMX-PROMOD- NCT-MIAS-12		
GeoMx Immune Cell Typing Panel Mouse Protein Module for nCounter	Protein module including 8 targets for mouse immune cell typing. Includes AbMix and Probe R4 for 48 AOI per slide. Must be run with a protein core. Currently available for pre-order. Shipment schedule will be confirmed at a later date.	12 slides	GMX-PROMOD- NCT-MICT-12		
GeoMx Pan-Tumor Panel Mouse Protein Module for nCounter	Protein module including 9 targets for mouse pan-tumor analysis. Includes AbMix and Probe R5 for 48 AOI per slide. Must be run with a protein core. Currently available for pre-order. Shipment schedule will be confirmed at a later date.	12 slides	GMX-PROMOD- NCT-MPT-12		
GeoMx Cell Death Panel Mouse Protein Module for nCounter	Protein module including 9 targets for human cell death. Includes AbMix and Probe R6 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-MCD-12		
GeoMx PI3K/AKT Signaling Panel Mouse Protein Module for nCounter	Protein module including 8 targets for human PI3K/AKT signaling. Includes AbMix and Probe R7 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-MPI3K-12		
GeoMx MAPK Signaling Panel Mouse Protein Module for nCounter	Protein module including 10 targets for human MAPK signaling. Includes AbMix and Probe R4 for 48 AOI per slide. Must be run with a protein core.	12 slides	GMX-PROMOD- NCT-MMAPK-12		

## **Ordering Information**

GeoMx Morphology Kits					
Product	Product Description	Quantity	Catalog Number		
GeoMx Solid Tumor TME Morphology Kit Human Protein Compatible	Morphology kit for visualization of human solid tumors and the tumor microenvironment. For use with protein assays. Includes fluorescent antibodies against Pan-CK, CD45, and a nuclear stain.	12 slides	GMX-PRO-MORPH- HST-12		
GeoMx Melanoma TME Morphology Kit Human Protein Compatible	Morphology kit for visualization of human melanoma and the tumor microenvironment. For use with protein assays. Includes fluorescent antibodies against S100B/PMEL17, CD45, and a nuclear stain.	12 slides	GMX-PRO-MORPH- HMEL-12		
GeoMx Solid Tumor TME Morphology Kit Mouse Protein Compatible	Morphology kit for visualization of mouse solid tumors and the tumor microenvironment. For use with protein assays. Includes fluorescent antibodies against Pan-CK, CD45, and a nuclear stain.	12 slides	GMX-PRO-MORPH- MST-12		
GeoMx Melanoma TME Morphology Kit Mouse Protein Compatible	Morphology kit for visualization of mouse melanoma and the tumor microenvironment. For use with protein assays. Includes fluorescent antibodies against S100B/Pmel17, CD45, and a nuclear stain.	12 slides	GMX-PRO-MORPH- MMEL-12		

Additional Assay Reagents					
Product	Product Description	Quantity	Catalog Number		
GeoMx Protein Slide Prep Kit for FFPE	Sample prep reagents for GeoMx DSP protein analysis. Includes Buffer W and Buffer S.	12 slides	GMX-PREP-PRO- FFPE-12		
GeoMx Hyb Code Pack Protein	nCounter readout reagents for GeoMx DSP protein analysis. Includes Hyb Code A-H, Probe U, and additional hybridization buffer.	96 AOI	GMX-PRO-HYB-96		
GeoMx DSP Collection Plate	Barcoded collection plates for use on the GeoMx DSP. Required for AOI tracking. Kit includes 4 plates covering 384 AOI.	1 Pack	GMX-DSP-COLL-PLT-4		
GeoMx DSP Instrument Buffer Kit	Buffer kit for the GeoMx DSP. Includes Buffer S and Buffer H. Sufficient for ~48 samples with ~18 AOI each. Volume requirements may vary based on experimental design.	1 Kit	GMX-DSP-BUFF-KIT		
nCounter Analysis System Master Kit Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing on the nCounter Analysis System.	96 AOI	NAA-AKIT-012		
nCounter SPRINT Cartridge	Sample Cartridge for nCounter SPRINT System	96 AOI	SPRINT-CAR-1.0		
nCounter SPRINT Reagent Pack	nCounter SPRINT Reagent Pack containing Reagents A, B, C, and Hybridization Buffer	1,536 AOI	SPRINT-REAG-KIT		

#### **Selected Publications**

- 1. Blank CU, et al. Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. Nat Med 2018; 24: 1655-1661.
- 2. Toki MI, et al. High-plex predictive marker discovery for melanoma immunotherapy treated patients using Digital Spatial Profiling. CCR 2019.
- 3. Wargo J, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med 2018; 24(11): 1649-54.

#### For more information, please visit nanostring.com/GeoMxDSP

NanoString Technologies, Inc. 530 Fairview Avenue North Seattle, Washington 98109

T (888) 358-6266 F (206) 378-6288

nanostring.com info@nanostring.com Sales Contacts United States us.sales@nanostring.com EMEA: europe.sales@nanostring.com

Asia Pacific & Japan apac.sales@nanostring.com Other Regions info@nanostring.com

nanoString

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

© 2021-2022 NanoString Technologies, Inc. All rights reserved. NanoString, NanoString Technologies, nCounter, GeoMx, and the NanoString logo are registered trademarks of NanoString Technologies, Inc., in the United States and/or other countries.

APR 2022 MK3350