GeoMx® Human Protein Assays

Spatially Profile Hundreds of Protein Targets with Next-Generation Sequencing Readout

Profile tens to hundreds of protein targets simultaneously with spatial resolution in any region of interest from a single tissue section using the GeoMx Digital Spatial Profiler (DSP). With a modular design, the GeoMx Human Protein Assays provide validated content for immunology, immuno-oncology, and neuroscience research.

Product Highlights

- Validated, multiplex antibodies designed for immunology, immuno-oncology, and neuroscience research
- Quantify hundreds of protein targets by selecting 10-plex modules to add to the GeoMx Human Protein Core
- Customizable with up to 10 additional antibodies of interest
- For use with Illumina next-generation sequencer (NGS) readout
- Utilize the GeoMx Data Center for interactive analysis

GeoMx Protein Assay Design

The GeoMx Human Protein Assays with NGS readout allow you to profile up to hundreds of protein targets simultaneously with spatial resolution using NGS platforms and pipelines. The four-plex GeoMx Protein Core for NGS, which includes necessary controls for GeoMx DSP experiments, can be run with any selection of 10-plex modules. GeoMx protein assays contain validated antibodies conjugated to unique DNA indexing-oligonucleotides via a UV-photocleavable linker. DNA oligonucleotide sequences contain region of interest (ROI) indices mapping them back to their tissue location, a protein target identification sequence matching them to their antibody, and a unique molecular identifier (UMI) to deduplicate reads. After selecting ROIs on GeoMx DSP, the DNA oligonucleotides are UV cleaved and then sequenced on an Illumina sequencer. Sequenced oligonucleotides are processed and then imported back into the GeoMx DSP platform for integration with the slide images and ROI selections for spatially-resolved protein expression.

Validated Content for Immunology, Immuno-Oncology, and Neuroscience

Immune	Oncology	Neuro	Human Protein Core for NGS			
+	+	+	Human Protein Core for NGS	Includes markers for immune cells (CD45), proliferation (Ki-67), antigen presentation (B2M), and vasculature (CD31), and the controls needed to run any GeoMx DSP experiment.	CD45	Rb IgG
					Ki-67	Ms IgG1
					Beta-2-microglobulin	Ms IgG2a
					CD31	Histone H3
						S6
						GAPDH
			Human Protein Modules for NGS - *Compatible with Illumina Systems*			
+	+		Immune Cell Typing	Includes key immuno-oncology targets and markers of immune cell types, including T cells, B cells, macrophages, NK cells, and stroma.	CD20	GZMB
					CD3	FOXP3
					CD4	CD34
					CD56	CD66b
					CD8	Fibronectin
+	+		Immune Activation Status	Includes additional checkpoint molecules and other markers of activated or memory T cells.	CD127	CD44
					CD25	CD27
					CD80	PD-1
					ICOS	PD-L1
					PD-L2	CD45RO
+	+		IO Drug Target	Includes drug targets in development within the immuno-oncology space, including checkpoint molecules and metabolic mediators of immune function.	4-1BB	B7-H3
					LAG3	IDO1
					OX40L	STING
					Tim-3	GITR
					VISTA	CTLA4
	+		Pan-Tumor	Includes markers for detecting EMT or cells of epithelial origin, and an expanded set of targets for detecting specific tumor types, including ER+/HER2+ breast tumors, hematopoietic malignancies, and melanoma.	MART1	PTEN
					NY-ESO-1	ER-alpha
					Bcl-2	PR
					EpCAM	PAN-CK
					Her2	SMA
+	+	+	Cell Death	Includes protein mediators of immunogenic and programmed cell death.	BAD	CD95/Fas
					BCL6	GZMA
					BCLXL	NF1
					BIM	p53
					Cleaved Caspase 9	PARP
+	+	+	MAPK Signaling	Includes key proteins involved in MAPK signal transduction, and phosphorylated protein products that measure pathway activation.	BRAF	pan-RAS
					p44/42 MAPK ERK1/2	EGFR
					Phospho-p44/42 MAPK ERK1/2 (T202/Y204)	Phospho-MEK1 (S217/S221)
					Phospho-JNK (T183/Y185)	Phospho-p38 MAPK (T180/Y182)
						Phospho-p90 RSK (T359 S363)

Immune	Oncology	Neuro	Human Protein Modules for NGS - *Compatible with Illumina Systems*			
+	+	+	PI3K/AKT Signaling	Includes key proteins involved in PI3K-AKT signal transduction, and phosphorylated protein products that measure pathway activation	Phospho-AKT1 (S473)	Pan-AKT
					Phospho-GSK3B (S9)	MET
					Phospho-GSK3A (S21)/Phospho-GSK3B (S9)	INPP4B
					Phospho-PRAS40 (T246)	PLCG1
					Phospho-Tuberin (T1462)	
+	+	+	Myeloid	Includes proteins expressed by myeloid cells generally or specific subsets, including macrophages, dendritic cells, and microglia.	HLA-DR	CD11b
					CD11c	CD14
					CD40	ARG1
					CD163	CD39
					CD68	
+	+	+	Autophagy	Includes proteins involved in the regulation and process of autophagy.	ATG12	LAMP2A
					ATG5	LC3B
					BAG3	P62
					GBA	TFEB
					HSC70	VPS35
		+	Neural Cell Typing	Includes relevant markers of neurons, oligodendrocytes, astrocytes, and microglia.	MAP2	IBA1
					Synaptophysin	P2ry12
					GFAP	TMEM119
					Myelin basic protein	NeuN
					Neurofilament light	Olig2
		+	Alzheimer's Disease Pathology	Includes proteins and protein products that are associated with Alzheimer's pathology and risk in the literature, including beta-amyloid, Tau, and ApoE.	Amyloid Precursor Protein	APOE
					Amyloid-Beta 1-40	P2RX7
					Amyloid-Beta 1-42	Tau
					Phospho-Tau (S404)	Tdp-43
					Phospho-Tdp-43 (S409/S410)	Ubiquitin
		+	Alzheimer's Disease Pathology Extended	Includes proteins and protein products that are associated with Alzheimer's pathology and risk in the literature, including increased coverage of phosphorylated Tau and amyloid processing proteins.	ADAM10	PSEN1
					BACE1	Phospho-Tau (T231)
					IDE	Phospho-Tau (S396)
					Neprilysin	Phospho-Tau (S199)
					NRGN	Phospho-Tau (S214)
		+	Parkinson's Disease Pathology	Includes proteins associated with Parkinson's pathology and risk in the literature, including several Parkin genes and alpha-synuclein.	Alpha-synuclein	FUS
					ApoA-I	LRRK2
					Calbindin	Park5
					Phospho-Alpha-synuclein (S129)	Park7
					Tyrosine Hydroxylase	PINK1
		+	Glial Cell Subtyping	Includes key markers of all glial cell subtypes, including microglia, astrocytes, and oligodendrocytes.	C4B	Mertk
					CD9	Vimentin
					CSF1R	Clec7a
					CTSD	Emp1
					GPNMB	S100B

Validated Assays Ready for Use

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

Ordering Screen
IHC validation of off-the-shelf antibody in control tissue(s)

Post-Conjugation Screen
IHC testing of conjugated antibody for post-conjugation specificity

Sensitivity/ Specificity Screen
Quantitative assessment on control FFPE cell pellets

Interaction Screen
Performance comparison of antibodies alone or in high-plex cocktail

TMA Screen
Analysis of normal and tumor tissue microarrays to confirm performance in tissue

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)**.

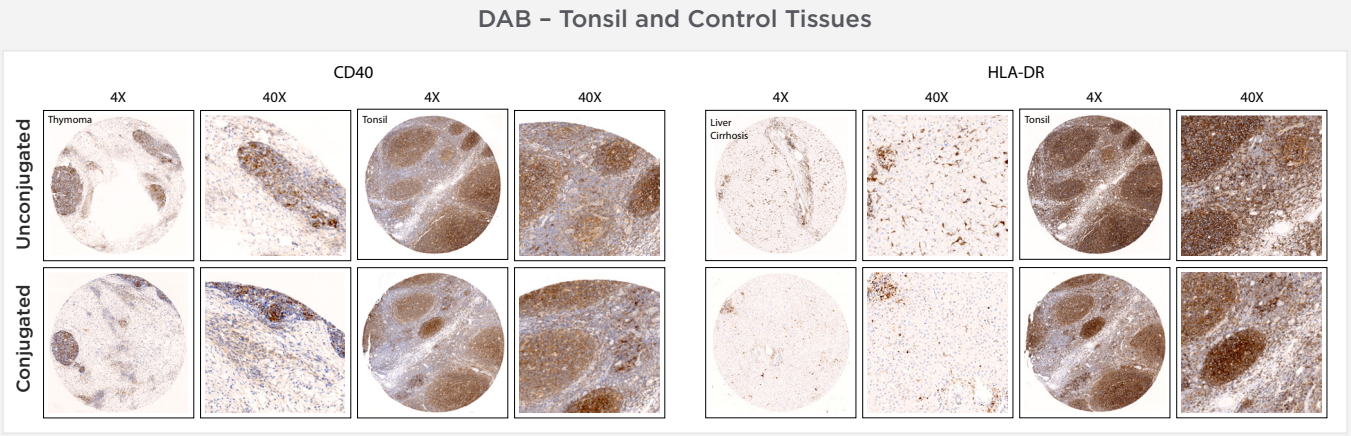

FIGURE 1: Example CD40 and HLA-DR from the GeoMx® Myeloid Module are tested for specific staining pre- and post-conjugation to a specific indexing-oligonucleotide to ensure conjugation does not alter specificity.

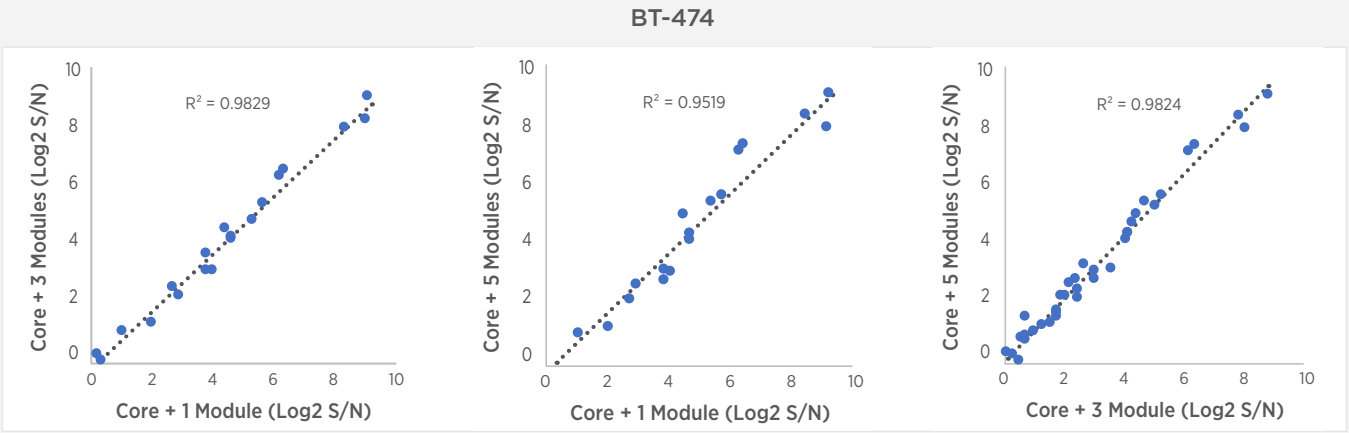

FIGURE 2: Example signal for the GeoMx® Human Protein Core for NGS is compared to the GeoMx® Human Protein Core for NGS plus individual Modules to ensure no antibody-antibody interference in BT-474 cell lines.

Reveal Tissue Heterogeneity

Analysis of mixed tumor and tumor microenvironment ROI from colorectal cancer (CRC) show distinct protein expression profiles in each segment respectively **(Figure 3)**.

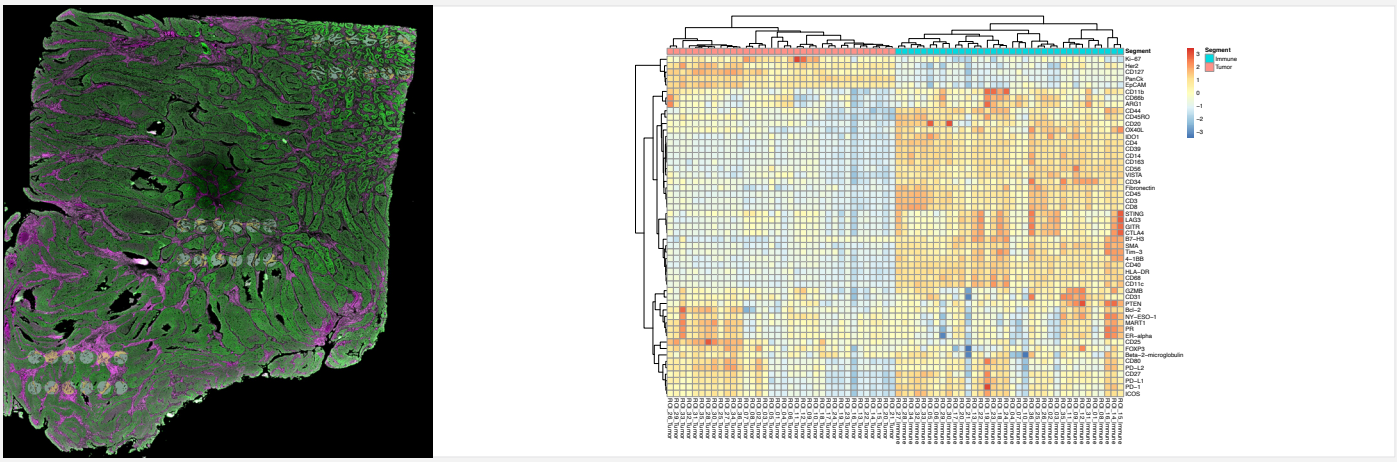

FIGURE 3: ROIs were selected with mixed tumor and tumor microenvironment (immune) segments in CRC FFPE tissue. ROIs were segmented based on PanCK/CD45 morphology stain. Protein expression shows strong clustering by compartment.

GeoMx® Data Analysis

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.

To view the Protein probe list, visit: nanostring.com/geomx-protein-assays

To view GeoMx publications, visit: nanostring.com/GeoMxPubs

Ordering Information

GeoMx Human Protein Assays for NGS			
Product	**Product Description**	**Quantity**	**Catalog Number**
GeoMx Human Protein Core for NGS*	Protein core including 4 targets for immune cells (CD45), proliferation (Ki-67), antigen presentation (B2M), and vasculature (CD31), plus positive and negative controls. Includes AbMix for Illumina NGS readout.	12 slides	GMX-PROCO-NGS-HCORE-12
GeoMx Immune Cell Typing Panel †	Protein module including 10 targets for human immune cell typing. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HICT-12
GeoMx IO Drug Target Panel †	Protein module including 10 targets for human immuno-oncology drug targets. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HIODT-12
GeoMx Immune Activation Status Panel †	Protein module including 10 targets for human immune activation status. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HIAS-12
GeoMx Pan-Tumor Panel †	Protein module including 10 targets for human pan-tumor analysis. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HPT-12
GeoMx Myeloid Panel †	Protein module including 9 targets for myeloid cells. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HMY-12
GeoMx MAPK Signaling Panel †	Protein module including 9 targets for human MAPK signaling. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HMAPK-12
GeoMx PI3K/AKT Signaling Panel †	Protein module including 9 targets for human PI3K/AKT signaling. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HPI3K-12
GeoMx Neural Cell Typing Panel †	Protein module including 10 targets for human neural cell typing. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HNCT-12
GeoMx Alzheimer's Pathology Panel †	Protein module including 10 targets for human AD pathology. Includes AbMix for Illumina NGS readout. Must be run with a protein core.	12 slides	GMX-PROMOD-NGS-HADP-12
GeoMx Morphology Kits			
GeoMx Solid Tumor TME Morphology Kit ‡	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 ‡	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 Alzheimer's Morphology Kit §	Morphology kit for visualization of human and mouse AD or other brain samples. For use with protein assays. Includes fluroescent antibodies against amyloid-beta, Iba1, and a nuclear stain.	12 slides	GMX-PRO-MORPH-HAD-12
GeoMx Parkinson's Morphology Kit §	Morphology kit for visualization of human and mouse PD or other brain samples. For use with protein assays. Includes fluroescent antibodies against alpha-synuclein, MAP-2, and a nuclear stain.	12 slides	GMX-PRO-MORPH-HPD-12
Additional Assay Reagents			
GeoMx Seq Code Pack†	NGS readout reagents for GeoMx DSP RNA and protein analysis. Includes two Seq Code primer plates (choice of A&B, C&D, E&F, or G&H) and two universal enzyme master mixes.	192 AOI	GMX-NGS-SEQ-AB
GeoMx Protein Slide Prep Kit	Sample prep reagents for GeoMx DSP protein analysis. Includes Buffer W and Buffer S.	12 slides	GMX-PREP-PRO-FFPE-12
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

* Compatible with Illumina Systems.
† Human Protein Module for NGS, compatible with Illumina Systems.
‡ Human Protein Compatible.
§ Human & Mouse Protein Compatible.

For more information, please visit nanostring.com/GeoMxDSP

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