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ROI Selection Markers

And CRO Services for GeoMx® Digital Spatial Profiling

ROI Selection Markers are designed for use with the GeoMx® Digital Spatial Profiler (DSP) platform from NanoString. The GeoMx DSP platform offers high-plex spatial profiling of protein or RNA targets. Morphology markers are fluorescently labeled antibodies used to select biologically relevant regions of interest (ROIs) for spatially guided analysis of transcriptional and proteomic pathways. Morphology Marker Kits from NanoString broadly identify solid tumors, but researchers have requested additional cell- and disease-specific markers. Canopy Biosciences is a leader in CRO services for GeoMx DSP assays and has developed a catalog of ROI Selection Markers, which are cell- and disease-specific. Our catalog includes pre-qualified markers as a supplement to Morphology Marker Kits from NanoString, and includes critical targets in immunology, oncology, and neuroscience to enable ROI selection with greater precision.

Research Applications

Our ROI Selection Markers are designed for a broad range of research applications in immunology, oncology, and neuroscience. The catalog includes markers relevant in recent literature and continues to expand by investigator request. This catalog focuses on key targets for immune cell profiling and immuno-oncology drug targets for:

- Cancer research aimed to profile molecular and cellular basis of cancer including tumor microenvironment, tumor evolution and response to treatment, and immune response to cancer
- Drug development and research including target selection and validation, preclinical toxicity studies, and pharmacodynamic studies

Product Highlights

- Designed for GeoMx® Digital Spatial Profiling Assays
- Compatible with Morphology Marker Kits from NanoString
- Pre-validated according to guidelines from NanoString
- We can validate new targets to create a fully custom set of markers

Preferred CRO Partner for GeoMx DSP Assays

The GeoMx DSP system from NanoString is a highly sensitive and widely adopted method for ROI-guided analysis of transcriptional and proteomic pathways. As a preferred CRO partner for GeoMx DSP services, we are uniquely positioned to offer custom target validation for ROI selection. This catalog significantly expands the current availability of markers for use with FFPE and fresh frozen tissues:

- Immune Cell Profiling
- Immune Cell Activation Status
- Immuno-oncology Drug Targets
- Neuronal Cell Profiling
- Neuro-oncology Drug Targets
- Lung Oncology Targets

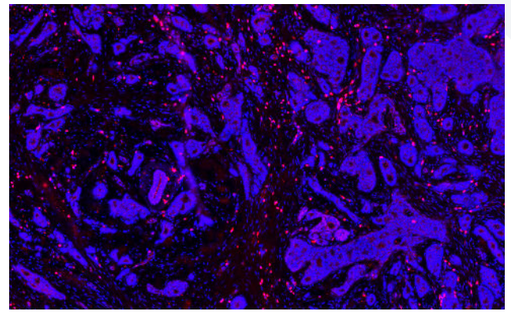

Figure 1. CD3 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

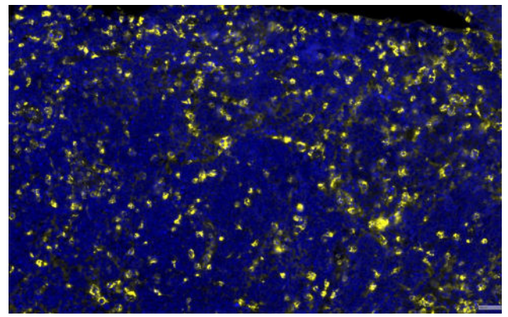

Figure 2. CD68 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

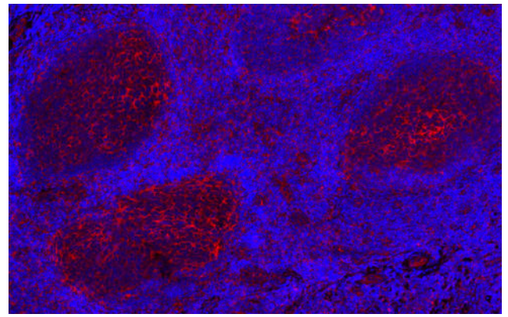

Figure 3. TIGIT expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

Marker Catalog Development

Using the Morphology Markers validation guidelines from NanoString, we qualify and verify antibodies for use in FFPE or fresh frozen tissue that target your protein of interest. We can test and validate antibodies for virtually any antigen for any tissue type using antibodies conjugated to fluorophores compatible with the GeoMx DSP system. Select from our growing catalog of pre-validated ROI Selection Markers for targeting proteins in human tissue samples.

Custom ROI Selection Markers

If your protein of interest is not available in this catalog, we can validate it for you. We continually test new targets for clients from any commercial vendor. Typically we focus on add-on markers to augment the off-the-shelf kits provided by NanoString, but our capabilities also include validating fully custom morphology marker sets.

For more information, email us at info.canopy@bruker.com

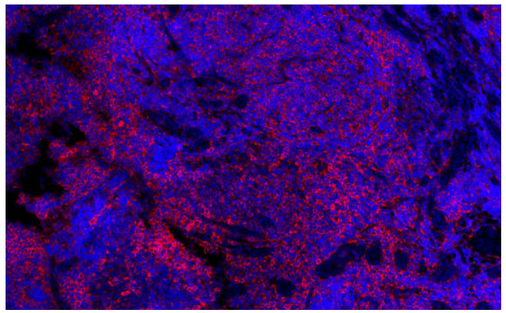

Figure 4. CD27 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

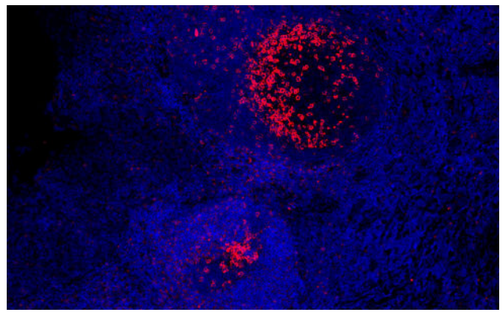

Figure 5. PD-1 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

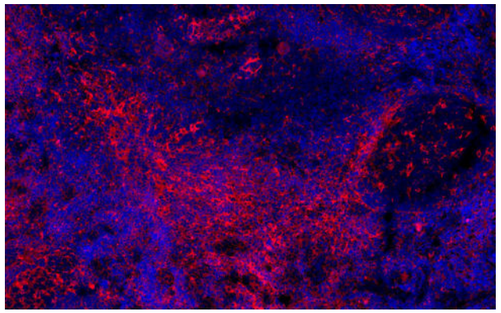

Figure 6. PD-L1 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

ROI Selection Marker Validation

Canopy Biosciences follows the same testing approaches for qualification and verification of Morphology Markers presented by NanoString. Canopy Biosciences® ROI Selection Markers undergo extensive validation to ensure high quality datasets for precise analysis.

Antibody Selection

Antibodies with compatible fluorophores are carefully selected from commercial vendors. Markers must be IHC-validated and be available in the open channel along with the main imaging channels for GeoMx DSP.

Antibody Testing

We use NanoString's Morphology Marker Guidelines as the basis for our process to qualify and verify morphology markers for use in GeoMx DSP. Markers are tested in control tissues as a first pass to determine staining quality and compatibility with conditions for the transcriptomic analysis workflow.

Qualification & Verification

Target-specific positive tissue staining is verified by an experienced pathologist and dilution is optimized to assess specificity and reduce background. Antibodies are also tested in combination with other markers in a multiplexed assay to rule out spectral interference.

Ongoing Evaluation

We are continuously evaluating markers for their suitability for staining new tissue types and performing ongoing optimization. Antibodies undergo testing on multiple tissues and are successfully used for ROI segmentation.

ROI Selection Marker List

Immune Cell Profiling: Critical markers to identify immune cell types, including T cells, B cells, macrophages, and NK cells. Additional markers for deeper profiling of immune cells, including subtyping T cells.

Immune Cell Profiling	
Marker	**Description**
CD3	Key marker of T-cells with a critical role in T-cell mediated responses
CD4	Key marker of helper T-cells with a critical role in adaptive immune response
CD11c	Key marker of dendritic cells, macrophages, and neutrophils
CD8	Key marker of cytotoxic T-cells with a critical role in targeted cell killing
CD45	Key marker of immune cells with a critical role in T-cell activation
CD68	Key marker of monocytes and macrophages
FoxP3	Key marker of regulatory T-cells with a critical role in cell development
Granzyme B	Key marker of cytotoxic T-cells and NK cells with a critical role in apoptosis

Immune Cell Activation: Critical checkpoint molecules that modulate T cell activation. Key markers of T cell activation mediate the progression of immune response.

Immune Cell Activation	
Marker	**Description**
CD27	Key marker of memory B cells with a critical role in T-cell proliferation
CD28	Key marker of activated T-cells with a critical role in T-cell proliferation
OX40	Key marker of activated T-cells, also expressed on NK cells, NKT cells, neutrophils
PD-L1	Key marker of activated T-cells and immune checkpoint inhibitor

Immuno-oncology Drug Targets: Critical drug targets in development in immuno-oncology, including many immune checkpoint molecules. Drug targets have the potential to enhance anti-cancer immune responses.

Immuno-oncology Drug Targets	
Marker	**Description**
B7-H4	Inhibitory receptor involved in T-cell activation and cytokine production
CTLA-4	Inhibitory receptor involved in T-cell activation in early immune response
IDO	Inhibitory enzyme involved in tryptophan and interleukin production
LAG-3	Inhibitory receptor involved in T-cell activation and effector functions
OX40L	Inhibitory receptor involved in DNA-binding transcription factor activity
PD-1	Inhibitory receptor involved in T-cell activation and apoptosis
TIGIT	Inhibitory receptor involved in T-cell activation and interleukin production
TIM-3	Inhibitory receptor involved in interleukin and interferon production

ROI Selection Marker List

Neuronal Cell Profiling: Critical markers to identify neuronal cell types including microglia, macrophages, and others. Additional markers for deeper profiling of cells involved in regulating neurotransmitter synthesis and ion transport.

Neuronal Cell Profiling	
Marker	**Description**
ChAT	Key marker of cholinergic neurons with a critical role in acetylcholine synthesis
DAT	Key marker of neuronal synapse with a critical role in dopamine transport
GAD67	Neuronal cell marker with a critical role in GABA synthesis
GFAP	Key marker of astrocytes with a critical role in cell development
MBP	Neuronal cell marker with a critical role in myelin formation and stabilization
Nestin	Key neuronal cell marker with a critical role in axon growth
NeuN	Neuronal cell marker with a critical role in pre-mRNA alternative splicing
Synaptophysin	Neuronal cell marker with a key role in synaptic plasticity
TH	Neuronal cell marker with a critical role in dopamine synthesis
TPH	Neuronal cell marker with a critical role in seratonin synthesis
VGLUT	Neuronal cell marker with a critical role in glutamate ion transport

Neuro-oncology Drug Targets: Critical drug targets in development and prognostic markers in neuro-oncology. Drug targets have the potential to enhance anti-cancer immune responses, while prognostic markers may help predict disease course.

Neuro-oncology Drug Targets	
Marker	**Description**
EGFR	Cell surface receptor with a key role in cell proliferation
FOXG1	Transcriptional regulator with a key role in neuronal cell differentiation
IDH	Mitochondrial enzyme with a key role in the generation of NADPH
L1CAM	Cell adhesion protein with a key role in migration and cell-cell communication
Olig2	Transcriptional regulator with a key role in cell differentiation
p53	Transcriptional regulator and tumor suppressor with a key role in cell survival
PTEN	Transcriptional regulator and tumor suppressor with a key role in cell survival
Sox2	Transcriptional regulator with a key role in cell differentation

ROI Selection Marker List

Lung Oncology Targets: Critical prognostic markers and drug targets in lung oncology. Drug targets have the potential to enhance anti-cancer immune responses, while prognostic markers may help predict disease course.

Lung Oncology Targets	
Marker	**Description**
ALK	Cell surface receptor and key marker in non-small cell lung cancer
INSM1	Transcriptional regulator and key marker in neuroendocrine tumors
MET	Cell surface receptor with a key role in cell proliferation
Napsin	Intracellular proteinase and key marker in lung adenocarcinoma
p40	Transcriptional regulator and key marker in squamous cell carcinoma
p63	Transcriptional regulator and key marker in small cell carcinoma
ROS1	Cell surface receptor and key marker in non-small cell lung cancer
TTF-1	Transcriptional regulator and key marker in lung adenocarcinoma

About Us

Canopy Biosciences is a global provider of products and services for accelerating multi-omics research. We acquire novel scientific instruments and offers CRO services for:

- ChipCytometry™
- Histopathology/IHC
- FISH analysis
- Single-cell and bulk RNAseq
- nCounter Analysis System
- GeoMx Digital Spatial Profiler

As a CLIA-certified laboratory, we are equipped to provide support for preclinical and clinical trial studies.

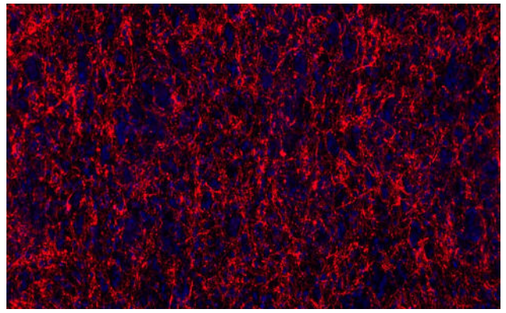

Figure 7. MPB expression in human glioma tissue using Canopy Biosciences® ROI Selection Markers.

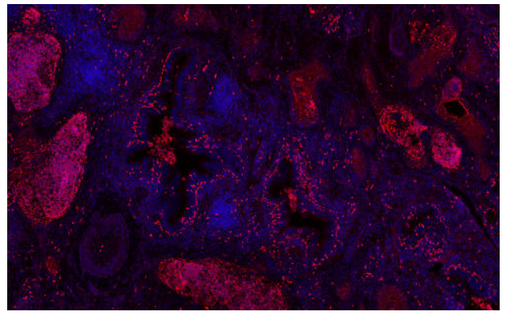

Figure 8. p63 expression in human lung tissue using Canopy Biosciences® ROI Selection Markers.

Contact Us for Custom Markers

If our catalog of ROI Selection Markers does not include your protein of interest, contact us for custom marker validation.

For custom projects, email us at info.canopy@bruker.com

To learn more, visit CanopyBiosciences.com/
or email us hello.canopy@bruker.com

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MARCH 2023 V2