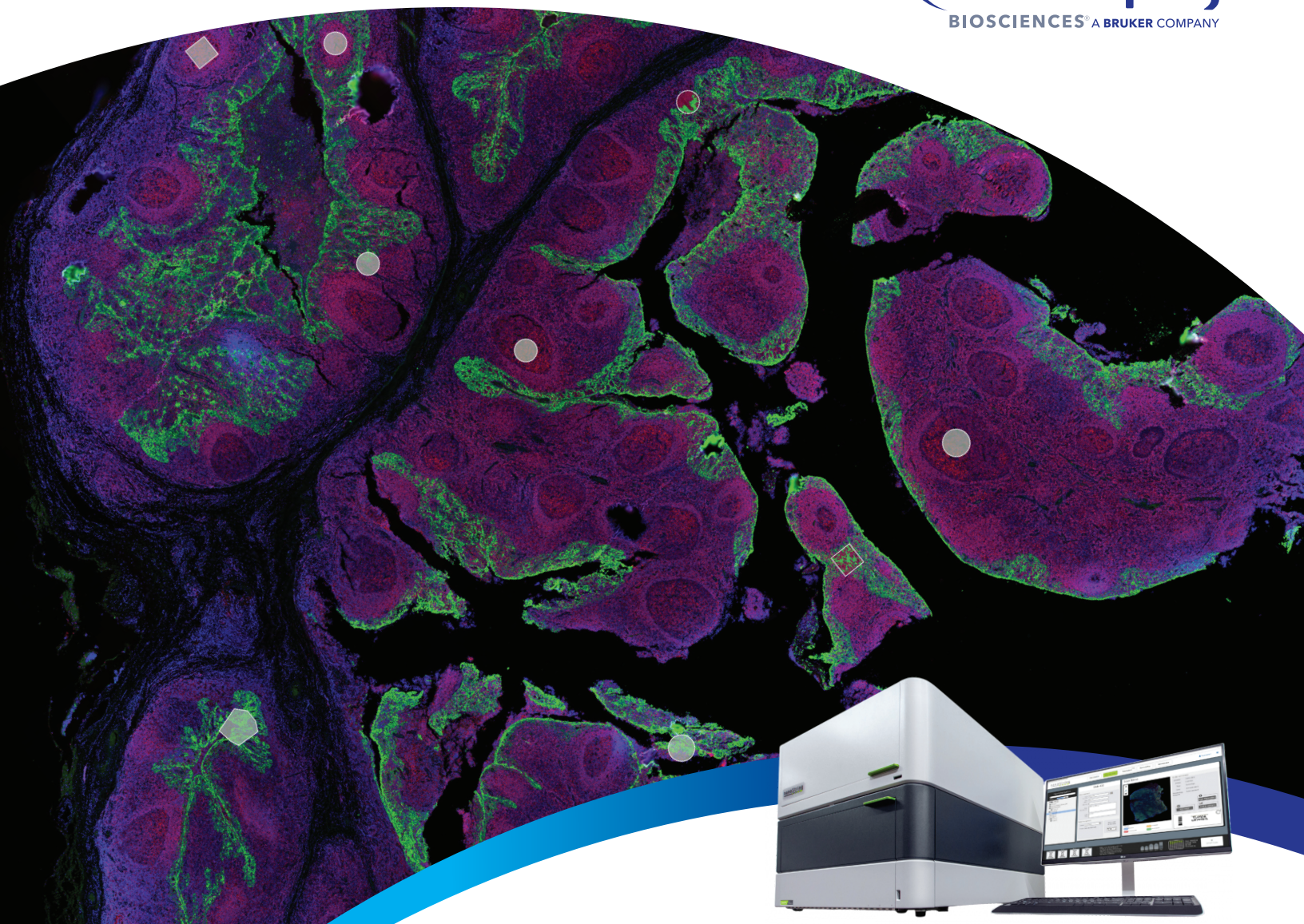
canopy BIOSCIENCES® A BRUKER COMPANY



Canopy Biosciences®

ROI Selection Markers

Supplemental Morphology Markers for NanoString® GeoMx® Digital Spatial Profiling Assays

Canopy Biosciences® ROI Selection Markers are designed for use with NanoString® GeoMx® Digital Spatial Profiling RNA and protein assays. NanoString GeoMx® offers high-plex spatial profiling of protein or RNA targets using fluorescently labelled antibodies as morphology markers. Morphology markers are used to select biologically relevant regions of interest (ROIs) for spatially guided analysis of transcriptional and proteomic pathways. Morphology Marker Kits from NanoString® are limited to a few markers that broadly identify solid tumors, but researchers have expressed a need to expand this to cell- and disease-specific markers. Canopy Biosciences® offers pre-qualified ROI Selection Markers as a supplement to Morphology Marker Kits from NanoString, to expand into critical targets in diverse applications including immuno-oncology and immunology to enable ROI-selection with greater precision.

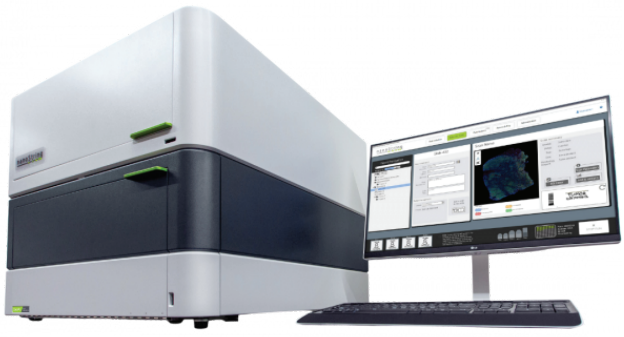
Research Applications

Canopy Biosciences® catalog of ROI Selection Markers is designed for a broad range of research applications in immunology and immuno-oncology and continues to expand into additional research applications based on investigator need. This catalog focuses on key targets for immune cell typing, immune cell activation status, 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 the Morphology Marker Guidelines from NanoString®
- Compatible with any tissue type including FFPE or fresh frozen tissue



Canopy Biosciences® ROI Selection Markers for GeoMx® Digital Spatial Profiling

NanoString® GeoMx® Digital Spatial Profiling Assays are highly sensitive and widely adopted method for the detection of protein and RNA targets. Morphology markers elucidate key structures and enable ROI-guided analysis of transcriptional and proteomic pathways. Canopy Biosciences® ROI Selection Markers enhance analysis by expanding availability of markers for ROI-selection. Markers for the following product categories are available for use with FFPE and fresh frozen tissues:

- Immune Cell Profiling
- Immune Cell Activation Status
- Immuno-oncology Drug Targets

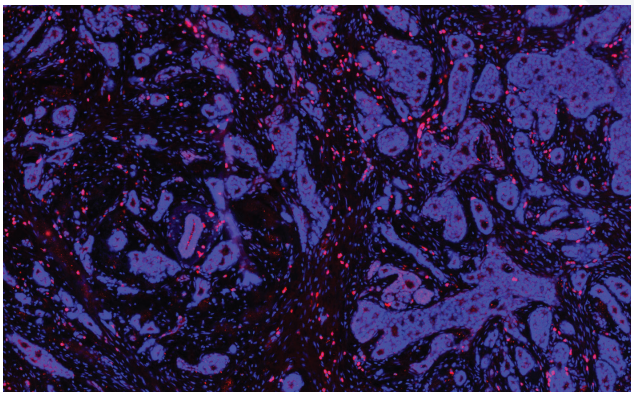


Figure 1. CD3 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

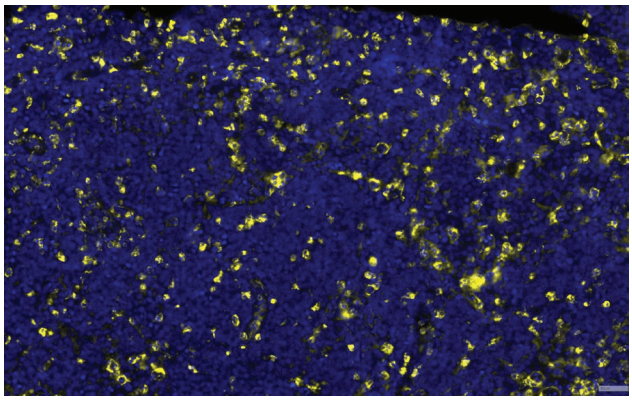


Figure 2. CD68 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

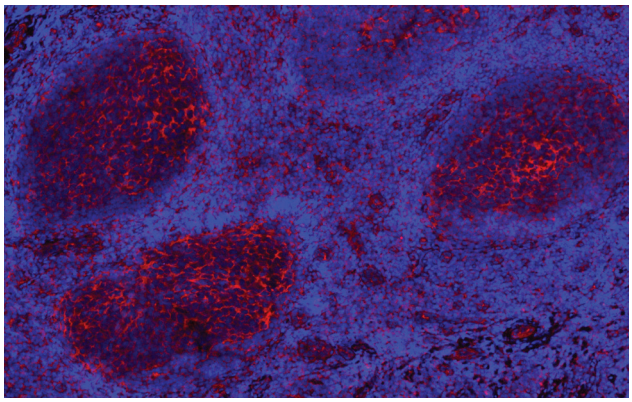


Figure 3. TIGIT expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

Canopy Biosciences® ROI Selection Markers

Using the NanoString® Morphology Markers guidelines, 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 NanoString® GeoMx® Digital Spatial Profiler system. Select from our growing catalog of pre-validated ROI Selection Markers for targeting proteins in human tissue samples.

Canopy Biosciences® Custom ROI Selection Markers

If Canopy Biosciences® catalog of ROI Selection Markers are not available for your protein of interest, we can test and validate new targets using antibodies from any commercial vendor to augment off-the-shelf Morphology Marker Kits from NanoString® or to create a fully custom set. Canopy Biosciences' method for marker validation can accommodate any antibody with a fluorophore conjugate compatible with GeoMx® Digital Spatial Profiler. Both catalog and custom markers are validated for use with and fully compatible with NanoString® Morphology Marker Kits.

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

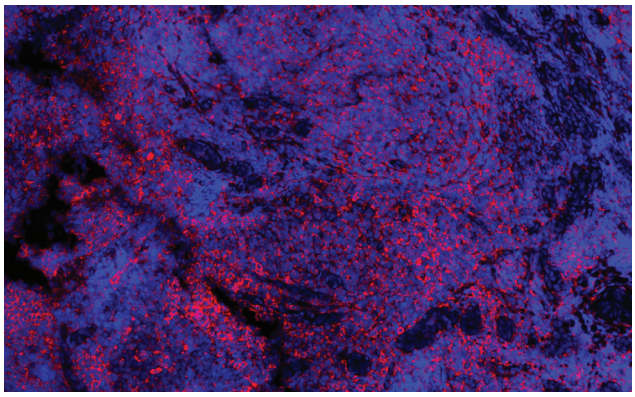


Figure 1. CD27 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

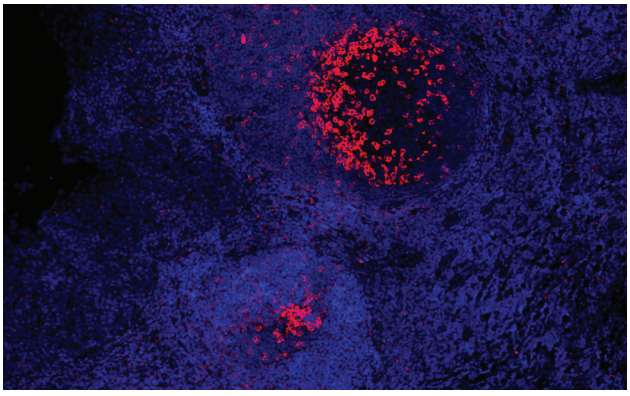


Figure 2. PD-1 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

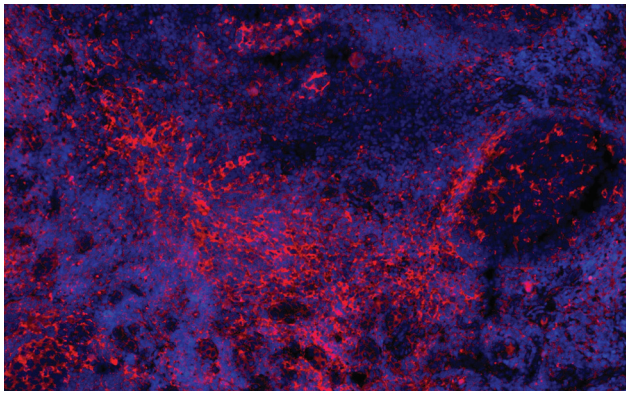


Figure 3. PD-L1 expression in human tonsil FFPE tissue using Canopy Biosciences® ROI Selection Markers.

Canopy Biosciences® 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

Canopy Biosciences® ROI Selection Markers are carefully selected antibodies with compatible fluorophore conjugates from commercial vendors to augment off-the-shelf kits. Markers are designed to work with both Morphology Marker Kits and NanoString® GeoMx® Digital Spatial Profiler to enhance ROI-guided analysis.

Antibody Testing

Canopy Biosciences® ROI Selection Markers are tested in control tissues as a first pass to determine staining quality and compatibility with conditions of subsequent transcriptomic analysis. Antibodies are also tested in additional tissues based on expected expression.

Qualification & Verification

Canopy Biosciences® ROI Selection Markers are qualified and verified based on Morphology Markers guidelines from NanoString®. Target-specific positive tissue staining is verified by an experienced pathologist and dilution is optimized to assess specificity and reduce background fluorescence.

Ongoing Evaluation

Canopy Biosciences® ROI Selection Markers are continuously evaluated for the suitability for staining new tissue types and ongoing optimization. Antibodies undergo testing on multiple tissues and are successfully used for ROI segmentation.

Canopy Biosciences® ROI Selection Markers

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 T cell marker with a critical role in T cell-mediated responses
CD4	Key T-helper cell marker with a critical role in adaptive immune response and T-cytotoxic cell recruitment
CD11c	Key dendritic cell marker, also expressed on monocytes, macrophages, and neutrophils, with a critical role in phagocytosis
CD8	Key T-cytotoxic cell marker with a critical role in targeted cell killing
CD68	Key monocyte and macrophage marker, with a critical role in apoptosis and phagocytosis
FoxP3	Key T-regulatory cell marker with a critical role in produc-tion of T-regulatory cells
Granzyme B	Key T-cytotoxic cell marker, also expressed on NK cells with a critical role in apoptosis

Immune Cell Activation Status: 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 critical role in T-cell proliferation and differentiation
CD28	Key marker of activated T cells and mediates signaling pathways for T-cell activation and survival, and cytotoxic secretion
OX40	Key marker of activated T cells, as well as NK cells, NKT cells, and neutrophils with a critical role in T cell survival and development of memory T cells
PD-L1	Key marker of activated T cells with a critical role in T cell survival and cytotoxic secretion

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

Immuno-oncology Drug Targets	
Marker	**Description**
B7-H4	Cell surface inhibitory receptor with influence over T cell activation, proliferation, and cytokine production
CTLA-4	Cell surface inhibitory receptor with influence over T cell activation in early immune response
IDO	Intracellular inhibitory enzyme with influence over tryptophan and interleukin production
LAG-3	Cell surface inhibitory receptor with influence over T cell activation and effector functions
OX40L	Cell surface inhibitory receptor with influence over DNA-binding transcription factor activity
PD-1	Cell surface inhibitory receptor with influence over T cell activation and apoptosis
TIGIT	Cell surface inhibitory receptor with influence over T cell activation and interleukin production
TIM-3	Cell surface inhibitory receptor with influence over interleukin and interferon production

Contact Us for Custom ROI Selection Markers

If Canopy Biosciences® catalog of ROI Selection Markers are not available for your protein of interest, contact us to initiate custom marker validation.

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

To learn more, visit canopybiosciences.com/geomx