APPLICATION NOTE

# Unravel the Complexity of Mouse Brain Tumors

## Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle

### Introduction

Brain neoplasms represent a complex form of cancer that is challenging to classify and treat. More than 120 tumor subtypes originate from various parts of the central nervous system, which makes identifying the composition of the tumor microenvironment (TME) vital for early assessment of progression, treatment and prevention[1]. Imaging Mass Cytometry™ (IMC™) technology offers unprecedented insight into the TME by simultaneously uncovering the spatial distribution of 40-plus distinct molecular markers without autofluorescence data artifacts, facilitating the research of brain neoplasms. Here, we demonstrate the application of a high-plex Maxpar™ OnDemand Mouse Neuro-Oncology IMC Bundle on normal and tumor FFPE mouse brain tissues (Figure 1). This panel consists of the validated Maxpar Neuro Phenotyping IMC Panel Kit (201337) and the Maxpar OnDemand Mouse Immuno-Oncology IMC Panel Kit (9100005), as shown in Table 1.

The Neuro Phenotyping IMC Panel consists of cross-reactive clones (human and mouse) and enables flexible panel design for brain-specific research goals, such as brain tumor classification and assessment of neuronal inflammation, activation and development. We applied the Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle on mouse normal brain and glioblastoma tissues. We successfully identified major cell populations that make up mouse brain matter, such as neurons, astrocytes, microglia and oligodendrocytes. Various tumor cell phenotypes and resident and infiltrating immune cells were detected in mouse glioblastoma TME.

### Objectives

- Showcase the performance of high-parameter IMC imaging on mouse brain tissues using the Maxpar Neuro Phenotyping IMC Panel Kit
- Illustrate the ability of the 35-marker Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle to identify immuno-oncological processes within the brain TME
- Exhibit the power of the panels to enable single-cell analysis for fast and accurate brain tumor and immune cell phenotyping

Subsequent single-cell analysis provided a comprehensive and quantitative assessment of the brain TME in mouse glioblastoma tissues. Empowered by the Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle, IMC technology can accelerate brain tumor research and provide insights into the spatial complexity of neuronal neoplasms.

## Study design

A high-parameter 40-marker antibody panel designed to highlight central features of the mouse neurological TME (Figure 1, Table 1) is presented in this application note. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle consists of the Maxpar Neuro Phenotyping IMC Panel Kit in combination with the Maxpar OnDemand Mouse Immuno-Oncology IMC Panel Kit. In addition, we used the Maxpar IMC Cell Segmentation Kit (201500) and Cell-ID™ Intercalator-Ir (201192A). This high-plex IMC panel was assembled to reveal critical insights about tissue structure and the functional state of cells in normal and tumor-containing brain tissues (Table 1).

Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle (9100005NO):

- **The Maxpar Neuro Phenotyping IMC Panel Kit** detects the underlying cellular and structural composition of normal and tumorous brain tissue in both mouse and human. Moreover, the antibody kit highlights major cell populations as well as their activated or senescent state.
- **The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle** detects activation of signaling pathways, metabolism and growth, metastatic potential in cancer cells, and the presence of lymphoid and myeloid cell subtypes of immune cell infiltrates and their functional state

Other components:

- The Maxpar IMC Cell Segmentation Kit and Cell-ID Intercalator-Ir were applied to facilitate single-cell analysis

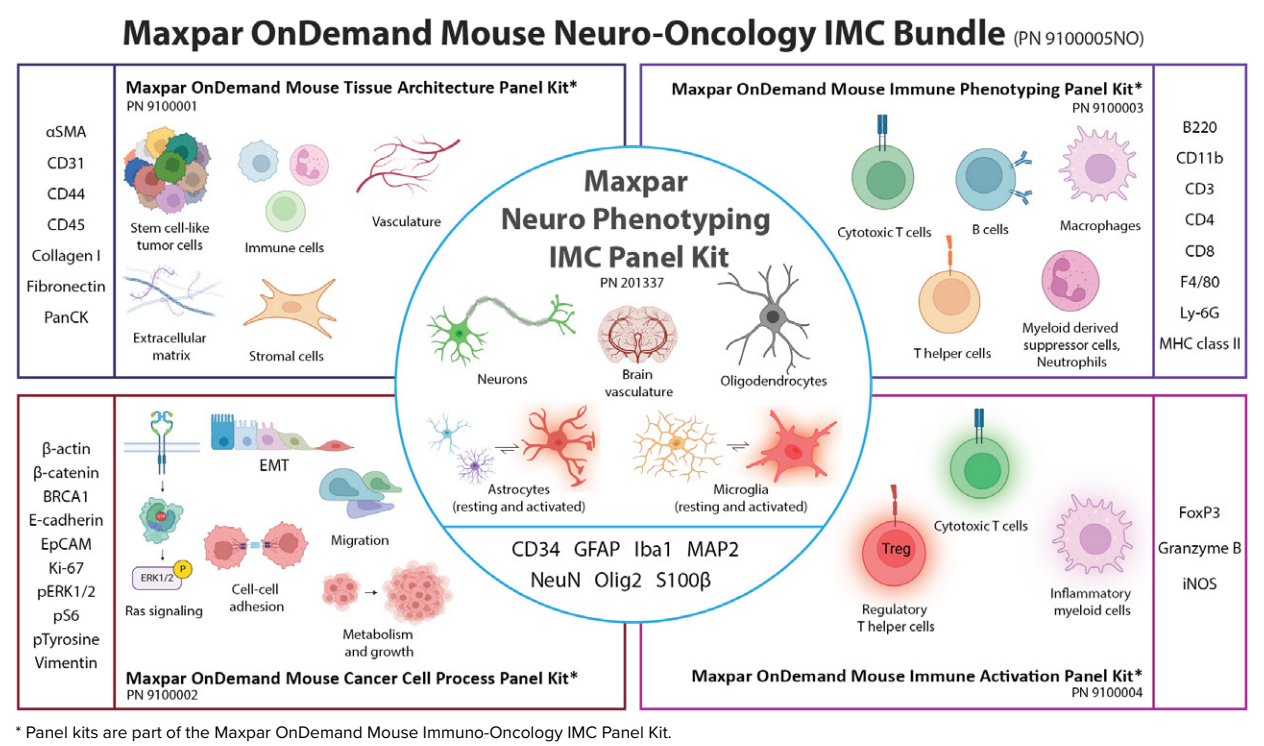


**Figure 1. Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle.** The panels are specifically designed to highlight the structure and cellular composition within the normal and diseased mouse brain TME. For additional details regarding specific metal combinations, antibody clones and targets, refer to Table 1.

The full panel was applied on sagittal sections of mouse normal (C57/BL6) brain and syngeneic glioblastoma (GL261) tissue. A variety of distinct sections of the normal brain (cerebral cortex, cerebellum, hippocampus) and glioblastoma (tumor core and margins) were ablated.

Normal and tumor tissue slides were prepared and stained using optimized antibody dilutions. The panel was titrated and tested on normal and tumor samples. Tissue slides were ablated using the Hyperion™ Imaging System. Qualitative data analysis, multiplexed image rendering and single-channel image extractions were performed using MCD™ Viewer. Quantitative single-cell analysis was performed for glioblastoma data using a pipeline consisting of 2 steps: CellProfiler was used for cell segmentation and histoCAT was used for t-distributed stochastic neighbor embedding (t-SNE) and PhenoGraph clustering. See Methods for additional experimental details regarding samples, staining, ablation and data analysis.

## Results

### Maxpar Neuro Phenotyping IMC Panel Kit detects the spatial position of major cell lineages in the brain

The brain is a complex organ composed of a vast array of cell types, each with its own unique function and role in brain physiology. Classifying major cell lineages in the brain is crucial to understanding the organization and function of its complex structure[1]. The Maxpar Neuro Phenotyping IMC Panel Kit combines markers for neurons, astrocytes, microglia, oligodendrocytes and endothelial cells, offering important insights into brain development and function (Figures 1 and 2).

In normal mouse tissues (cerebral cortex, cerebellum, hippocampus), NeuN labels neuronal cell bodies and MAP2 highlights axonal projections within the white matter. Astrocytes expressing GFAP are also abundantly found. S100β-expressing astrocytes, Iba1-expressing microglia and Olig2-expressing oligodendrocytes are dispersed in the brain tissue. CD34 highlights the presence of large and small blood vessels.

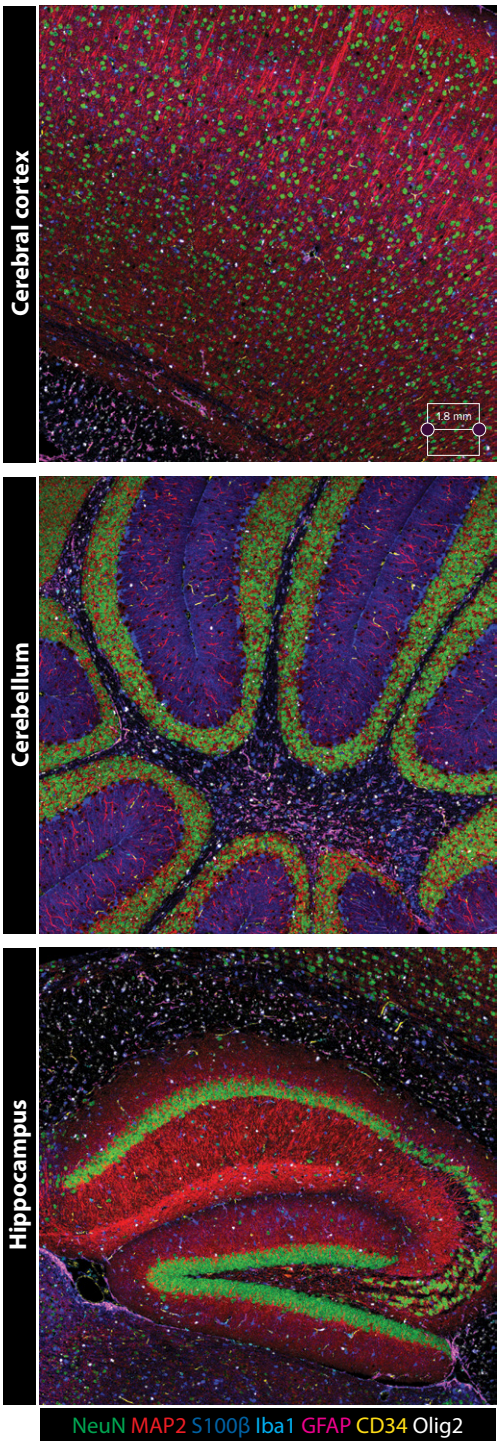


**Figure 2. Maxpar Neuro Phenotyping IMC Panel Kit identifies distinct spatial positioning of the major brain cell lineages in normal mouse brains.** Rendered images of FFPE mouse brain tissues demonstrate the performance of markers with virtually no background, typically observed in brain tissues due to autofluorescence. Scale bar applies to all images in the figure.

## Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle deciphers the tumor and immune cell populations in mouse glioblastoma TME

Glioblastoma is a highly complex and aggressive type of brain cancer. The highly heterogeneous tumor tissue contains diverse zones with varying genetic, molecular and cellular profiles that promote resistance to conventional therapies and tumor recurrence. Better understanding of the complex biology of glioblastoma is necessary to develop more effective treatments[2]. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle offers a wide variety of markers for detailed assessment of the brain TME.

### Assessment of tumor origin, stemness, and aggressiveness

Glioblastoma arises from glial precursor cells such as astrocytes and oligodendrocytes in the brain[3]. The Neuro Phenotyping IMC Panel Kit includes markers that help determine the cellular origins of any glioblastoma tissue. Assessment of the mouse glioblastoma tissue revealed the presence of Olig2 expression in tumor cells[4] (Figures 3 and **S1**). Expression of cancer stem cell (CSC) marker CD44 was detected in tumor cells, indicating the ability of tumor cells to self-renew and differentiate into potential metastatic cell types[5]. Expression of tumor differentiation biomarkers S100β, β-catenin and Ki-67 was noted in most tumor cells,

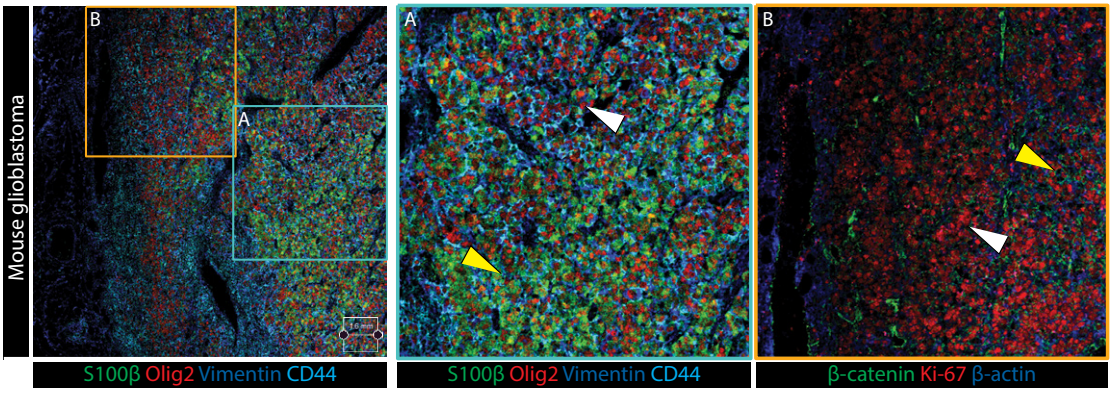


**Figure 3. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle provides assessment of cellular origin, stemness and differentiation of glioblastoma tumor cells.** Tumor cells of oligodendrocyte precursor cell lineage expressing Olig2 are detected (inset A, white arrowhead). Stem cell-like tumor cells expressing CD44 are observed (A). Tumor cells expressing S100β at high (inset A, yellow arrowhead) and low levels (inset A, white arrowhead) are present. β-catenin (inset B, yellow arrowhead) and Ki-67 (inset B, white arrowhead) define activated tumor cells undergoing cell replication. Vimentin (A) and β-actin (B) expression highlights the structural features of the tumor cell cytoskeleton.

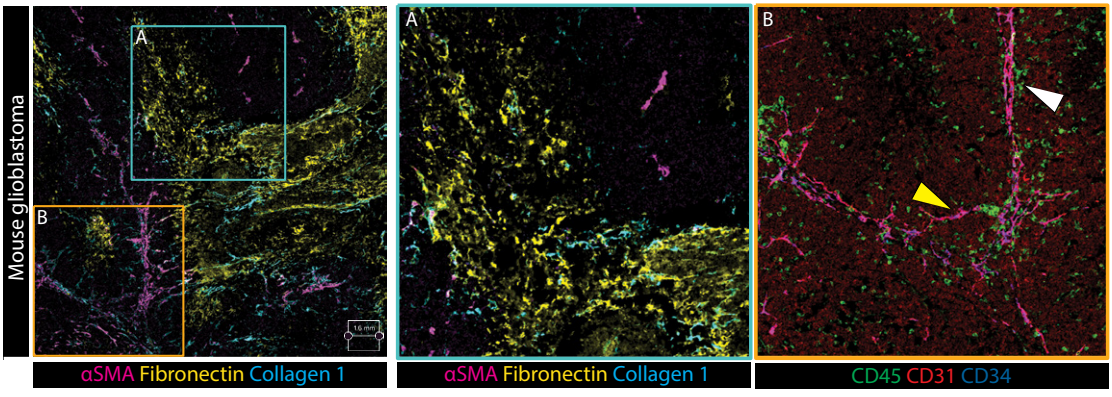


**Figure 4. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle provides assessment of tissue architecture in glioblastoma.** Fibronectin and collagen 1 markers offer insights into the positioning and composition of the extracellular matrix (inset A). αSMA labels vasculature-associated pericytes and stromal cells (inset A). Endothelial cell markers CD34 and CD31 evaluate the vascular coverage of the TME (inset B, yellow arrowhead). CD45 markers permit detection of immune cells (inset B, white arrowhead).

demonstrating the aggressiveness of the tumor. Accumulation of intermediate (vimentin+) and actin (β-actin+) filaments in tumor cells demonstrates the ability of tumor cells to migrate and metastasize from the primary tumor site.

### Extracellular matrix and vasculature

Glioblastoma exhibits abnormal distribution of extracellular matrix (ECM), which can act as a physical barrier to drug delivery and provide a protective environment for tumor cells[6]. ECM markers fibronectin and collagen 1 offer a detailed spatial assessment of ECM composition (Figures 4 and **S1**). The vasculature of glioblastoma undergoes faulty angiogenesis (the formation of new blood vessels from pre-existing ones) and forms leaky blood vessels that permit increased intravasation of tumor cells, extravasation of immune cells and accumulation of fluid in the brain[7,8]. Vascular markers αSMA, CD34 and CD31 permit a detailed assessment of vascular density and function. The accumulation of pan-immune cell marker CD45-expressing cells adjacent to blood vessels highlights extravasating immune cells.

### Signaling and metabolism

Targeting signaling pathways that promote cell proliferation and tumor survival is an active area of research with a goal to develop small molecular inhibitors to curb tumor growth[9]. Phosphorylated tyrosine (p-tyrosine) and pERK1/2 markers assess the activation of receptor tyrosine kinase (RTK) and Ras signaling pathways. Both markers are readily expressed in glioblastoma tissue (Figures 5 and S1). In glioblastoma, activation of the mTOR pathway, which regulates metabolic activation in tumor cells, is frequently observed[10]. Phosphorylated ribosomal subunit 6 (pS6) serves as a marker for increased metabolic activity, and its presence is detected in glioblastoma tumor cells.

### Immune cell infiltration

The tumor immune microenvironment (TIME) in glioblastoma is a highly dynamic system composed of lymphoid and myeloid cells that influence tumor growth and progression. Infiltration of lymphoid cells such as cytotoxic T cells, T helper cells and B cells is often associated with improved survival in patients with glioblastoma[11]. Myeloid cells, including macrophages and microglia, are also present in high numbers in glioblastoma TIME and are indicative of an immunosuppressive phenotype[12] (Figures 6 and **S1**). In mouse glioblastoma, specific T cell phenotypes are detected by utilizing the differential expression of CD3, CD8, CD4 and B220 markers. Myeloid cells are identified using microglial marker Iba1, macrophage marker F4/80, monocyte marker CD11b and neutrophil marker Ly-6G.

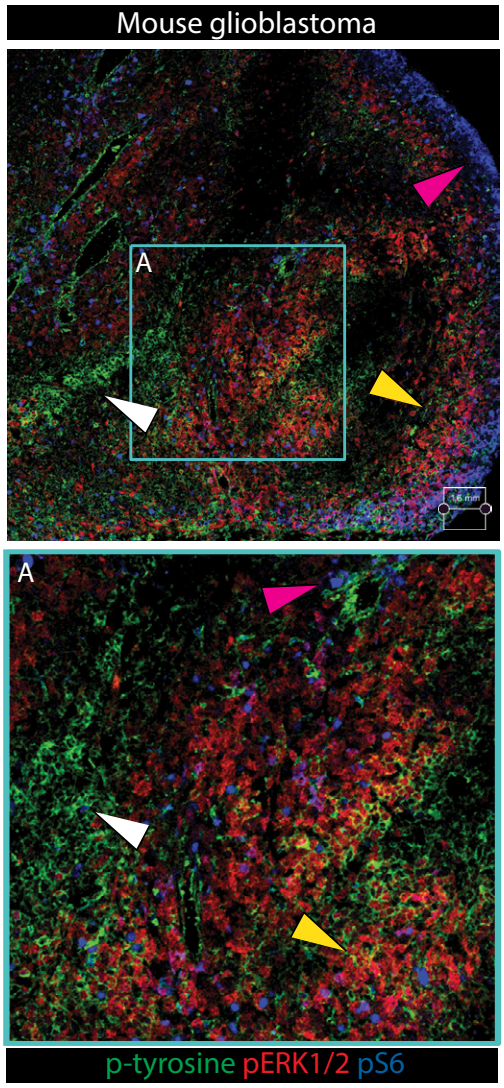


**Figure 5. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle provides assessment of signaling and metabolic pathway activation in glioblastoma.** The presence of p-tyrosine signal identifies cells with activated RTK-mediated signaling (inset A, white arrowhead). pERK1/2 expression indicates cells with stimulated mitogen-activated protein kinase (MAPK) signaling (inset A, yellow arrowhead). pS6, a component of the mTOR signaling pathway, highlights metabolically active cells (inset A, magenta arrowhead).

### Immune cell activation

Assessment of immune cell activity is important in designing effective immunotherapeutic treatments. Presence of cytotoxic T cells and regulatory T cells provides important information about the state of the TIME[13]. In mouse glioblastoma, the cytotoxic activation marker granzyme B is detected in CD8+ T cells and macrophages (Figure 7). Regulatory T cells expressing the FoxP3 marker are identified in the TIME.

## Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle enables single-cell analysis of mouse glioblastoma

In combination with the Maxpar IMC Cell Segmentation Kit, the Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle facilitates single-cell analysis of the mouse TME for the evaluation of tumor progression. Single-cell analysis of mouse glioblastoma detected a total of 139,847 cells from 5 tumor cores (2.5 $mm^2$ each). PhenoGraph analysis grouped the cells into 23 distinct clusters, with 12 designated as tumor cell-specific, 8 as immune cell-specific, 1 as vascular-specific and 2 as unspecified. Each cluster phenotype is defined by the presence or absence of a combination of markers from the antibody bundle (Figures 8 and **S2**).

### Senescent tumor cells

Clusters 1, 4 and 12 highlight senescent tumor cells that collectively make up 25% of the cells in the TME. Tumor cells in cluster 1 express Olig2 and vimentin, suggestive of non-replicative migratory cells. Cells in clusters 4 and 12 display high levels of stem cell marker CD44, indicative of high tumor stemness and the presence of a heterogeneous TME. Notably, stem cell-like tumor cells in cluster 12 are embedded in the ECM due to their spatial localization within fibronectin-enriched areas of tumor tissue.

### Activated tumor cells

Tumor-specific cell clusters 2, 7, 8 and 13 exhibit elevated levels of Ki-67. Collectively, these replicating cells represent 28% of the cells in the TME. Clusters 7 and 8 contain cells with elevated levels of S100β and pERK1/2, respectively, indicating activation of cell differentiation processes. Cluster 13 demonstrates increased signal for the mTOR signaling pathway component pS6, suggesting high cellular metabolic activity.

### Immune cells

Immune cells represent 12% of the total population of the TME with myeloid cells being the predominant type (77% of total immune cell population). All immune cell clusters exhibit expression of CD45 and enrichment of β-actin signal, suggestive of high migratory capacity. Cluster 9 encompasses resident microglia (Iba1+). Cluster 14 contains macrophages (F4/80+, CD11b+, MHC class II+) expression whereas cluster 16 comprises macrophages embedded in the ECM (fibronectin+, collagen 1+). Cluster 15 is composed of cytotoxic T cells (CD3+, CD8+). Cluster 17 contains regulatory T cells (CD3+, CD4+, FoxP3+), whereas cluster 22 contains T helper cells (CD3+, CD4+).

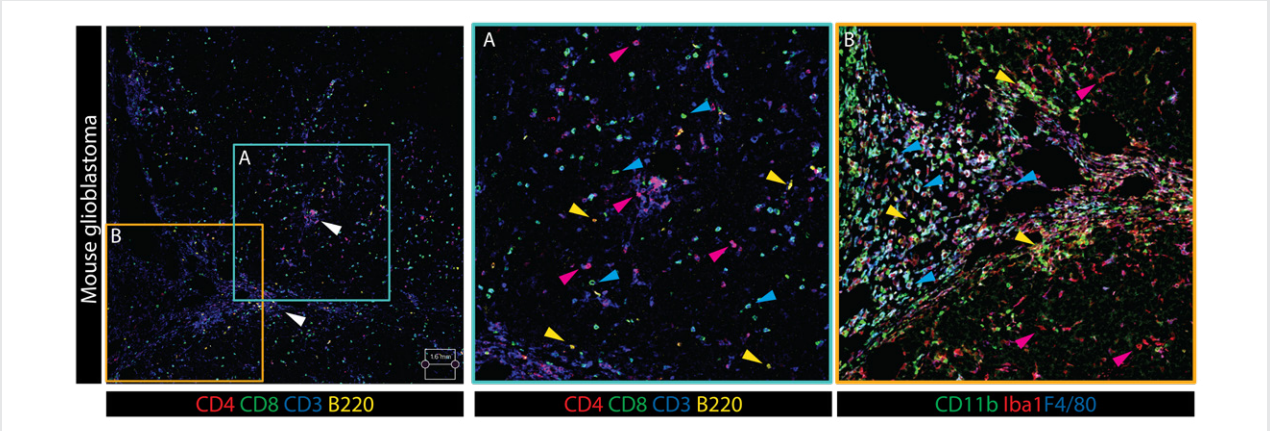


**Figure 6. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle provides an assessment of immune cell composition in the TME.** Lymphoid cells are identified by the expression of CD3 (T cells, white arrowheads) or B220 (B cells; inset A, yellow arrowheads). T cells are further subdivided into specific cell lineages through the assessment of CD4 (T helper cells; inset A, magenta arrowheads) and CD8 (cytotoxic T cells; inset A, blue arrowheads) markers. Infiltrating myeloid cells are identified by combinatorial expression of F4/80 (inset B, blue arrowheads) and CD11b (inset B, yellow arrowheads). Exclusive Iba1 expression highlights resident tissue microglia (inset B, magenta arrowheads).

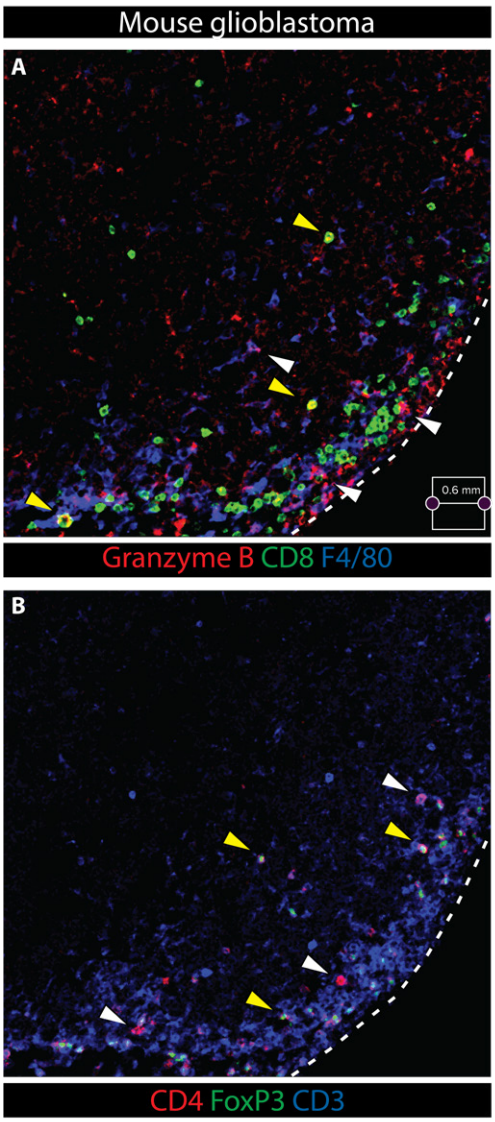


**Figure 7. The Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle provides an assessment of immune cell activation in the TME.** Activated cytotoxic T cells (CD3+) are identified by simultaneous expression of CD8 and granzyme B (A, yellow arrowheads). Activated cytotoxic macrophages are identified by simultaneous expression of F4/80 and granzyme B (A, white arrowheads). Exhausted regulatory T cells (CD4+, FoxP3+; B, yellow arrowheads) are distinguished from T helper cells (CD4+; B, white arrowheads) through expression of FoxP3. Scale bar applies to both images in the figure. Dashed line demarcates tumor margin.

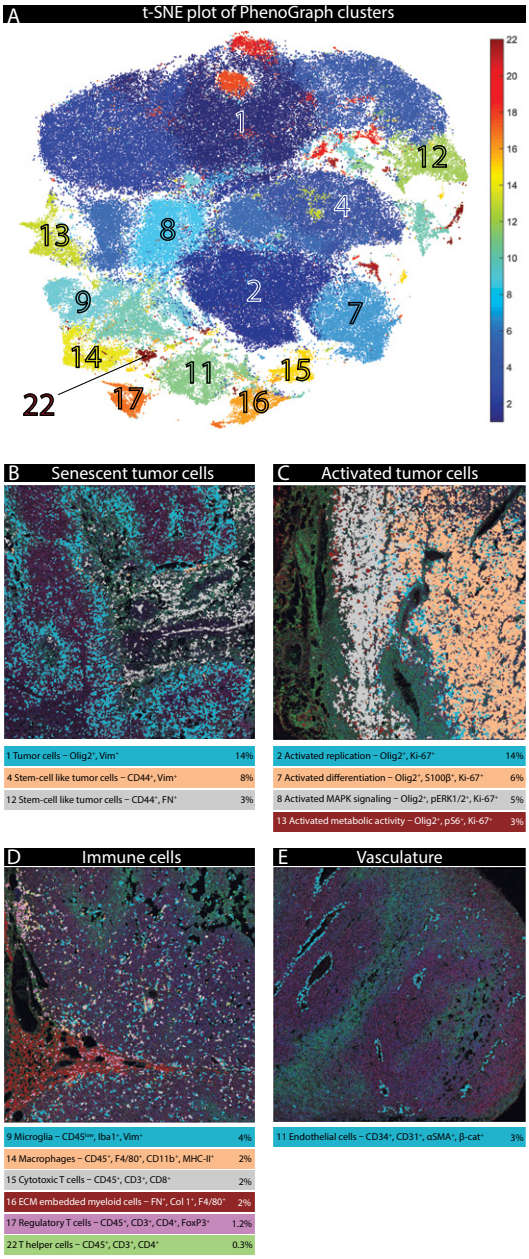


**Figure 8. Single-cell analysis of mouse glioblastoma reveals the cellular composition of the TME.** The t-SNE plot illustrates 23 cellular clusters identified by PhenoGraph analysis. Spatial positioning of the 14 clusters labeled in the t-SNE plot (A) is shown overlaid on glioblastoma tissues (B–E). Clusters are identified by marker expression along with the percent composition of cells in the TME. Cell masks are overlaid on images demonstrating ICSK1 (green), ICSK2 (red) and ICSK3 (blue). Vim, vimentin; FN, fibronectin; Col 1, collagen 1; β-cat, β-catenin; β-act, β-actin

### Vasculature

Vascular cells, identified by CD34 and CD31 signal and represented by cluster 11, make up 3% of the total cell population. High expression of vimentin was detected in the vascular cells, which suggests activation of vascular remodeling. In addition, enriched levels of β-catenin and extensive αSMA+ pericyte coverage suggest strong intercellular junctional integrity in blood vessels[14].

## Conclusions

Despite significant progress in preclinical cancer therapeutics, the lack of comprehensive data on their effects on neurological tumors has been a major challenge in their successful translation to the clinic. IMC technology offers a unique opportunity to evaluate up to 40-plus clinically relevant biomarkers while eliminating false positive background signal typically observed in brain tissues due to autofluorescence.

As described in this application note, the application of the Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle enables researchers to conduct detailed mouse neuro-oncology studies that can uncover crucial insights into tumor development, progression and treatment. Our quantitative analysis of mouse glioblastoma revealed insights regarding overall cellular composition of the TME and tumor prognostic parameters such as:

- Identity of tumor cell origin and stemness
- Activation of cellular processes in tumor cells
- Presence of resident and infiltrating immune cells
- Stimulation of immune cell activity
- Quantitative assessment of tumor tissue composition

Overall, the innovative Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle, including the human and mouse cross-reactive Maxpar Neuro Phenotyping IMC Panel Kit, facilitates accurate deciphering of complex biological processes, providing translational researchers and clinicians with a powerful tool to advance the understanding of brain neoplasms and improve patient outcomes.

## Tips for success

- For best results, use freshly cut FFPE tissue samples when possible
- Perform a 3-point titration and include positive control tissue for all antibodies when optimizing working concentration on tumor tissue. Recommended dilution ranges for each antibody can be found in the technical datasheet (TDS-00721).
- After staining, samples should be stored at room temperature in slide holders inside a sealed bag in a non-humid environment
- Customers should reach out to their local Field Applications Scientist (FAS) for ordering and product support. To be connected to a FAS, **contact technical support**.

## Methods

### Panel kit design

Antibodies were selected based on the best fit for the neuro-oncology application on tissues and full compatibility with the Maxpar OnDemand Mouse Immuno-Oncology IMC Panel Kit. For ordering information, refer to Table 1.

### Tissue

Mouse normal brain tissue sections (sagittal cut) were obtained from AMSBIO and mouse syngeneic glioblastoma (GL261) full tissue sections were obtained from Charles River and stored according to the manufacturer's recommendation before use. Several regions of the normal brain were ablated, including cerebellum, cerebral cortex and hippocampus. Varying regions of interest (ROI) were selected on glioblastoma tissue, including tumor cores and margins.

### Staining

Imaging was performed using the Hyperion Imaging System with CyTOF™ Software v7.0. Before ablation, instrument tuning was performed using a tuning slide. For normal and tumor tissue imaging, each ROI of 1.6 $mm^2$ or 2.56 $mm^2$* was selected and ablated at 200 Hz with 3 dB laser power and 1 µm resolution. For normal tissue, 3 ROIs shown in Figure 2 were ablated. For glioblastoma, 5 ROIs were selected from various locations on the tissue including tumor margins and cores. Single-channel channel images were exported from MCD files and used for subsequent analysis.

### Data analysis

MCD Viewer v1.0.560.6 (Standard BioTools) was used to render multiplexed and single channel 16-bit TIFF images. For qualitative verification of staining, images for each channel were rendered and verified to ensure absence of nonspecific and background staining. For glioblastoma tissue, raw single channel OME-TIFF files were exported for further analysis. Graphics shown in Figure 1 were created using biorender.com.

### Cell segmentation

The Maxpar IMC Cell Segmentation Kit and Cell-ID Intercalator-Ir were used to label the cell membrane and nuclei of all cells present in the TME, respectively. CellProfiler v4.2.1 was used to perform cell segmentation. A basic pipeline for cell segmentation was assembled, which included primary (nuclei) and secondary (cell membrane) object identification modules. Images containing individual cell masks were generated and extracted for single-cell analysis.

### Single-cell analysis

Single-channel OME-TIFF and cell masks for glioblastoma ROIs were loaded into histoCAT v1.76. t-SNE analysis and PhenoGraph clustering were performed. Masks representing specific clusters were plotted onto ROIs rendered with ICSK channels and cell quantities for each cluster were extracted and documented. All clusters were plotted on the t-SNE graph (Figure 8).

* The recommended maximum ROI size for the Hyperion Imaging System is 1.5 x 1.5 mm (2.25 $mm^2$), however it is possible to acquire larger ROIs. The ROI size for brain tissue was assigned to capture the optimal structure of the tissues. Please reach out to your local FAS for best practices regarding ROI selection.

| Products | | Metal | Marker | Clone | Target/Cellular Process | Part No. |
|---|---|---|---|---|---|---|
| Maxpar Neuro Phenotyping IMC Panel Kit (201337) | | 142Nd | Iba1 | EPR16588 | Microglia | 3142020D |
| | | 143Nd | GFAP | GA-5 | Astrocytes | 3143030D |
| | | 145Nd | NeuN | EPR12763 | Neurons (nuclei) | 3145019D |
| | | 146Nd | S100β | EP1576Y | Glial cells | 3146021D |
| | | 148Nd | MAP2 | EPR19691 | Neurons | 3148023D |
| | | 167Er | CD34 | EP373Y | Vascular cells | 3167025D |
| | | 168Er | Olig2 | EPR2673 | Oligodendrocytes | 3168028D |
| Maxpar OnDemand Mouse Immuno-Oncology IMC Panel Kit (9100005) | Maxpar OnDemand Mouse Tissue Architecture IMC Panel Kit (9100001) | 141Pr | αSMA | 1A4 | Smooth muscle/stromal cells | 3141017D |
| | | 171Yb | CD31 | EPR17259 | Vascular cell | 91H027171 |
| | | 153Eu | CD44 | IM7 | Tumor cell/immune cells | 3153029D |
| | | 151Eu | CD45 | D3F8Q | Immune cells | 91H029151 |
| | | 173Yb | Collagen 1 | Polyclonal | Extracellular matrix | 91H018173 |
| | | 152Sm | Fibronectin | EPR19241-46 | Extracellular matrix | 91H028152 |
| | | 174Yb | Pan-cytokeratin | AE-1/AE-3 | Cytoskeletal filament | 91H006174 |
| | Maxpar OnDemand Mouse Cancer Cell Process IMC Panel Kit (9100002) | 154Sm | β-actin | 2F1-1 | Cytoskeletal microfilament | 3154021D |
| | | 169Tm | β-catenin | 5H10 | $Ca^{2+}$ dependent cell adhesion | 91H022169 |
| | | 172Yb | BRCA1 | MS110 | Tumor suppressor | 3172030D |
| | | 158Gd | E-cadherin | 24E10 | $Ca^{2+}$ dependent cell adhesion | 3158029D |
| | | 147Sm | EpCAM | EPR20532-222 | $Ca^{2+}$ independent cell adhesion | 91H024147 |
| | | 150Nd | Ki-67 | B56 | Proliferating cells | 91H017150 |
| | | 164Dy | pERK1/2 | D13.14.4E | Ras signaling activation | 91H039164 |
| | | 175Lu | pS6[S235/S236] | N7-548 | mTOR pathway activation | 3175031D |
| | | 144Nd | p-tyrosine | P-Tyr-100 | RTK* activation | 3144024D |
| | | 149Sm | Vimentin | D21H3 | Mesenchymal cells | 91H002149 |
| | Maxpar OnDemand Mouse Immune Phenotyping IMC Panel Kit (9100003) | 176Yb | B220 | RA36B2 | B cells | 91H036176 |
| | | 163Dy | CD11b | EPR1344 | MDSCs†, M1 macrophages | 91H007163 |
| | | 170Er | CD3 | Polyclonal (C-term) | Pan T cells | 3170019D |
| | | 159Tb | CD4 | BLR16J | T helper cells | 91H031159 |
| | | 162Dy | CD8 | EPR21769 | Killer T cells | 91H023162 |
| | | 156Gd | F4/80 | D2S9R | Macrophages | 91H030156 |
| | | 166Er | Ly-6G | 1A8 | MDSCs, neutrophils | 91H037166 |
| | | 161Dy | MHC class II | M5/114.15.2 | Antigen-presenting cells | 91H038161 |
| | Maxpar OnDemand Mouse Immune Activation IMC Panel Kit (9100004) | 165Ho | FoxP3 | FJK-16s | Regulatory T cells | 91H032165 |
| | | 155Gd | Granzyme B | EPR22645-206 | Cytotoxic immune cell activation | 91H026155 |
| | | 160Gd | iNOS | SP126 | Activated macrophages | 91H025160 |
| Cell-ID Intercalator-Ir‡ | | 191Ir | DNA1 | | DNA | 201192A |
| | | 193Ir | DNA2 | | | |
| Maxpar IMC Cell Segmentation Kit‡ | | 195Pt | ICSK1 | | Cell membrane | 201500 |
| | | 196Pt | ICSK2 | | | |
| | | 198Pt | ICSK3 | | | |

* Receptor tyrosine kinase
† Myeloid-derived suppressor cells
‡ Cell-ID Intercalator-Ir and the Maxpar IMC Cell Segmentation Kit are not part of the Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle.

**Table 1. Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle (9100005NO) for FFPE brain tissue application**

## Required reagents

| Standard BioTools | Part Number |
|---|---|
| Maxpar PBS | 201058 |
| Cell-ID Intercalator-Ir | 201192A |
| Maxpar Water | 201069 |
| Maxpar antibodies | Multiple |
| Maxpar OnDemand Antibodies | Multiple |
| Maxpar IMC Cell Segmentation Kit | 201500 |

| Third-Party Reagents | Product Name | Part Number |
|---|---|---|
| Sigma-Aldrich | m-Xylene ReagentPlus | 185566-1L |
| Commercial Alcohols | Anhydrous ethyl alcohol | P006EAAN |
| Agilent | Antigen Retrieval Solution pH 9 (10x) | S236798-2 |
| Thermo Scientific | Triton X-100 | 85111 |
| Sigma-Aldrich | 10% Bovine Serum Albumin | A3059 |
| Charles River | Mouse FFPE tumor | Custom order |
| AMSBIO | Mouse FFPE normal brain tissue | 7011-0220-5PK |

## References


1. Sampson, J.H. et al. “Brain immunology and immunotherapy in brain tumors.” *Nature Reviews Cancer* 20 (2020): 12–25.
2. Campos, B. et al. “A comprehensive profile of recurrent glioblastoma.” *Oncogene* 35 (2016): 5,819–5,825.
3 Alcantara Llaguno, S.R. et al. “Adult lineage-restricted CNS progenitors specify distinct glioblastoma subtypes.” *Cancer Cell* 28 (2015): 429–440.
4. Liang, Y. et al. “Implantation of GL261 neurospheres into C57/BL6 mice: A more reliable syngeneic graft model for research on glioma-initiating cells.” *International Journal of Oncology* 43 (2013): 477–484.
5. Biserova, K. et al. “Cancer stem cells: Significance in origin, pathogenesis, and treatment of glioblastoma.” *Cells* 10 (2021): 621.
6. Henke, E. et al. ”Extracellular matrix in the tumor microenvironment and its impact on cancer therapy.” *Frontiers in Molecular Biosciences* 6 (2019): 160.
7. Arvanitis, C.D. et al. “The blood-brain barrier and blood-tumour barrier in brain tumors and metastases.” *Nature Reviews Cancer* 20 (2020): 26–41.
8. Pacheco, C. et al. “Glioblastoma vasculature: From its critical role in tumor survival to relevant *in vitro* modelling.” *Frontiers in Drug Delivery* 2 (2022).
9. Pearson, J.R.D. et al. “Targeting cellular pathways in glioblastoma multiforme.” *Signal Transduction and Targeted Therapy* 2 (2017): 17040.
10. Akhavan, D. et al. “mTOR signaling in glioblastoma: lessons learned from bench to bedside.” *Neuro-Oncology* 12 (2010): 882–889.
11. Han, S. et al. “Tumour-infiltrating CD4(+) and CD8(+) lymphocytes as predictors of clinical outcome in glioma.” *British Journal of Cancer* 110 (2014): 2,560–2,568.
12. Ye, Z. et al. “Phenotypic plasticity of myeloid cells in glioblastoma development, progression, and therapeutics.” *Oncogene* 40 (2021): 6,059–6,070.
13. Yang, I. et al. “CD8+ T-cell infiltrate in newly diagnosed glioblastoma is associated with long-term survival.” *Journal of Clinical Neuroscience* 17 (2010): 1,381–1,385.
14. Bergers, G. et al. “The role of pericytes in blood-vessel formation and maintenance.” *Neuro-Oncology* 7 (2005): 452–464.

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**Unravel the Complexity of Mouse Brain Tumors Application Note**

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