Evaluation of IDH1, EGFR, IGF-1R and Ki67 biomarkers in glioblastoma using the Lunaphore COMET™ platform

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INTRODUCTION

Glioblastoma (GBM) is the most aggressive primary neuroepithelial tumor diagnosed in the United States with approximately 14,000 diagnoses each year. Patients with GBM have a poor prognosis and only 5% of these patients survive for more than 5 years (2). A deeper understanding of the pathology of GBM has the potential to improve early diagnosis and treatment options for patients. Diagnosis of GBM requires tumor biopsies with consideration of histopathological and molecular characteristics (2). Specific markers involved with the histopathology of glioblastomas include Isocitrate Dehydrogenase 1 (IDH1), Epidermal Growth Factor Receptor (EGFR), Insulin Like Growth Factor 1 Receptor (IGF-1R), and a cell proliferating marker, Ki67 (3). Additionally, EGFR & IGF-1R are important markers as mutations or amplifications of these targets are present in more than 80% of primary glioblastomas (4). Pathogenic mutations also occur in most low-grade gliomas and secondary high-grade gliomas for IDH1, making this another important marker for glioblastoma (5). Typically, IHC is done using a single-biomarker HRP-DAB detection technique which is not suitable for the simultaneous spatial analysis of multiple biomarkers.

The complex nature of the tumor microenvironment (TME) makes spatial multiplex immunohistochemistry (IHC) and immunofluorescence (IF) ideal for visualizing biomarker expression and cellular localization. Antibody specificity, sensitivity, and availability for multiplex IF is a critical success factor for TME characterization. Emerging automated staining and imaging platforms rely on validated antibodies for accurate marker detection. Lunaphore COMET™ is a fully-automated, high-throughput, hyperplex platform for spatial analysis of Formalin Fixed Paraffin Embedded (FFPE) and frozen tissues. It performs sequential immunofluorescence (seqIF™) assays through staining, imaging, and elution cycles (Figure 1) (6). COMET™ utilizes unconjugated primary antibodies and fluorescently-conjugated secondary antibodies. We sought to validate GBM biomarkers IDH1, EGFR, IGF-1R and Ki67 for use on COMET™ to understand biomarker expression in GBM a the single-cell level.

MATERIALS & METHODS

Antibody Qualification: Protocol optimization to reach the best staining quality while assessing specificity, sensitivity, and elution efficiency.

1. R&D Systems™ IHC antibodies selected based on IF performance with manual staining on A172 GBM cell line (Figure 2).
2. Specificity is confirmed by comparing staining on a positive tissue sample, visual confirmation of subcellular localization, and overall expression pattern within the tissue.
3. Sensitivity is determined by staining tissues with different expression levels of the target protein.

Multiplex IF Protocol:

1. 5µm thick FFPE tissue sections of GBM were deparaffinized by incubating into a xylene bath followed by rehydration in decreasing concentrations of ethanol.
2. Antigen retrieval was performed in PT module with basic (pH9) retrieval buffer for 60 min at 102°C;
3. Slides with tissue sections and reagents were loaded and automated cycles of seqIF™ were run on COMET™.
4. One cycle of seqIF™ consists of primary antibody incubation, secondary antibody incubation, and imaging. Antibodies are then eluted using a gentle and efficient elution buffer ensuring optimal tissue preservation.
5. Images are registered by COMET™ Control Software at the end of the protocol execution and saved as single OME-TIFF file.

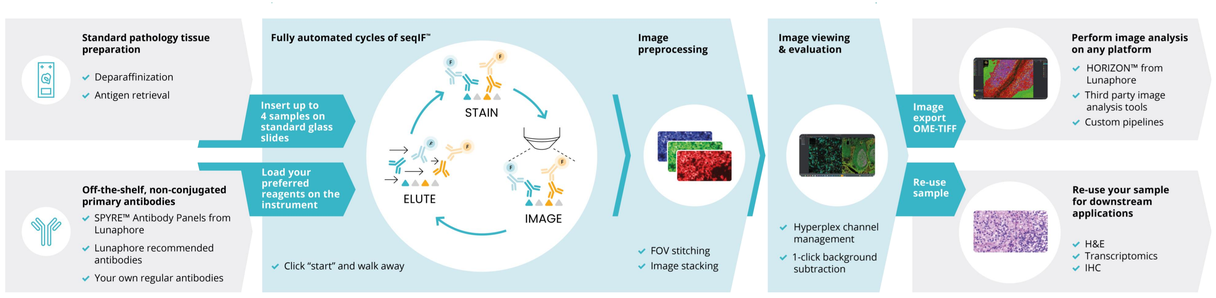


Figure 1. Overview of automated seqIF™ workflow on COMET™.

Primary Antibody	Concentration	Catalog Number
Mouse Anti-Human Isocitrate Dehydrogenase 1/IDH1	15 µg/mL	MAB7049, R&D Systems, a Bio-Techne Brand
Rabbit Anti-Human/Mouse Human Ki67/MKI67	5 µg/mL	MAB7617, R&D Systems, a Bio-Techne Brand
Goat Anti-Human EGFR	5 µg/mL	AF231, R&D Systems, a Bio-Techne Brand
Mouse Anti-Human/Mouse IGF-1 R/IGF1R	15 µg/mL	MAB391, R&D Systems, a Bio-Techne Brand
Secondary Antibody	**Dilution**	**Catalog Number**
Anti-Mouse Alexa Fluor® Plus 647	1:200	DR647MS, Lunaphore, a Bio-Techne Brand
Anti-Rabbit Alexa Fluor® Plus 555	1:100	DR555RB, Lunaphore, a Bio-Techne Brand
Anti-Goat Northern Lights 557	1:200	NL001, R&D Systems, a Bio-Techne Brand

Table 1. Primary and secondary antibodies

RESULTS

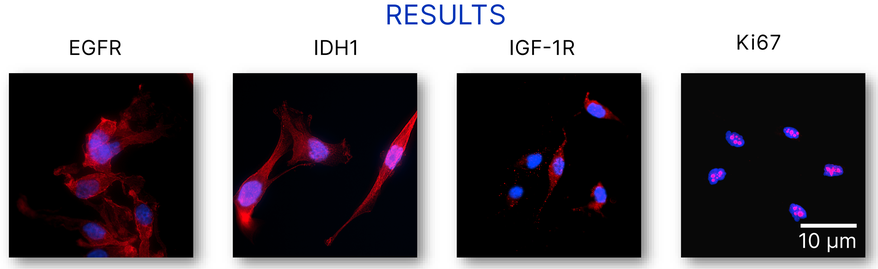
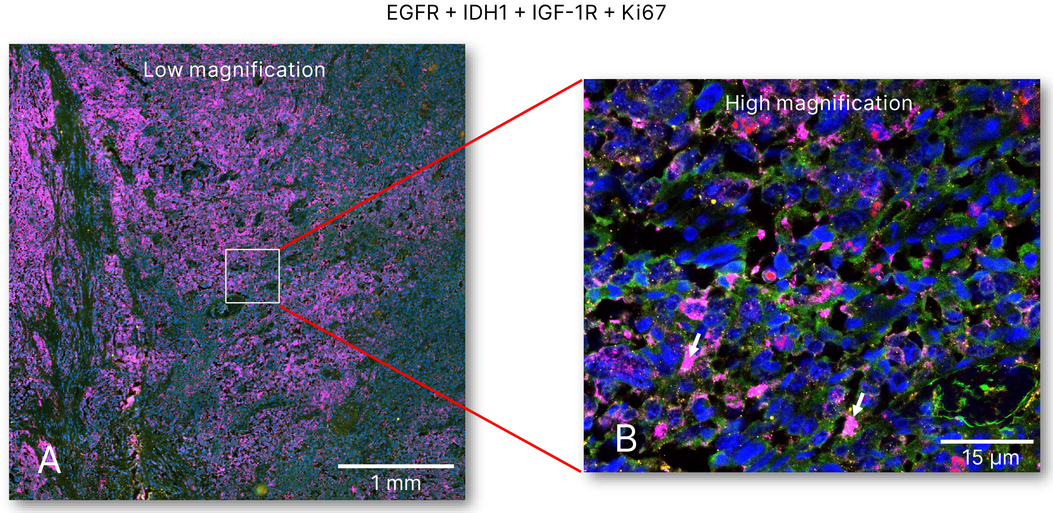


Figure 2. Initial validation of primary antibodies on A172 glioblastoma cell line.



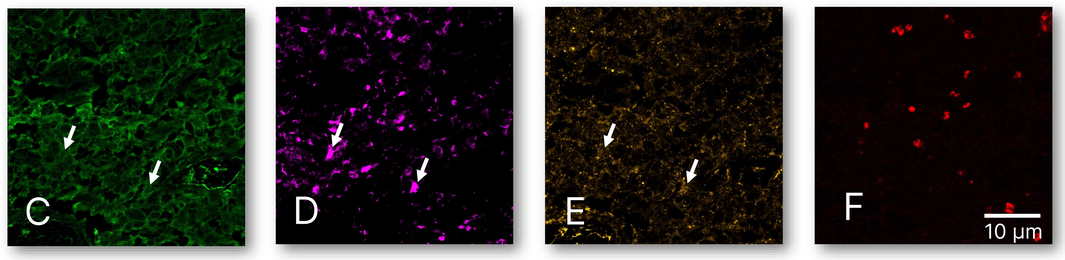


Figure 3. Spatial distribution of EGFR, IDH1, IGF-1R, and Ki67 in GBM on COMET™. FFPE GBM tissue section was stained with antibodies targeting EGFR (Green), IDH1 (Pink), IGF-1R (Yellow), Ki67 (Red), and DAPI (Blue) according to materials and methods. A) Pseudocolored, low magnification overview of stained tissue. B) Exploded high-magnification view of the area outlined by white square box. C – F) Single FL images of each marker. White arrows indicate coexpression.

CONCLUSIONS

1. Spatial codetection of multiple cancer biomarkers in brain allows for accurate analysis at the single cell level.
2. The Lunaphore COMET™ platform allows for an unambiguous distinction between IDH1, EGFR, IGF-1R and Ki67 in FFPE GBM tissue sections using commercially available, unconjugated primary antibodies.
3. Antibody qualification on COMET™ is critical to ensuring reliable and accurate staining.
4. Selecting COMET™ qualified antibodies from Bio-Techne streamlines custom panel development.

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