Aug 27, 2025 Version 1

Carver et al., Aged Brain Spatial Profiling - GeoMx V.1

In 1 collection

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Protocol status: Working
We use this protocol and it's working

Created: October 04, 2023

Last Modified: August 27, 2025

Protocol Integer ID: 88806

Keywords: aged brain spatial profiling, digital spatial profiling of mouse brain, modifiable features of aged brain white matter, aged brain white matter, geomx whole transcriptome atlas for mouse, old brain accumulation of lipofuscin granule, associated microglia, geomx digital spatial profiling machine platform, old brain accumulation, staining process for geomx, geomx whole transcriptome atlas, digital spatial profiling machine platform, digital spatial profiling, mouse brain, transcriptome in situ hybridiazation, fluorophore, autofluorescent noise, autofluoresce, lipofuscin granule

Abstract

This protocol provides the staining process for GeoMx digital spatial profiling of mouse brain used in in Carver et al., "Senescent- and disease-associated microglia are modifiable features of aged brain white matter". T

Sections were cut at 5 µm from FFPE blocks.

Brain slices were adhered to glass slides and stained directly on the surface

The immunostaining process involves unconjugated- or fluorophore-conjugated- primary antibodies and secondary antibodies conjugated to fluorophores. Due to old brain accumulation of lipofuscin granules that autofluoresce, we note that in order to achieve maximal signal, a quenching step is performed to extinguish the autofluorescent noise.

Transcriptome in situ hybridiazation was performed with the GeoMx Whole Transcriptome Atlas for mouse.

Sections were imaged and processed on the GeoMx digital spatial profiling machine platform.

GeoMx slide preparation

1   For FFPE sections, follow procedures found in document

📄 MAN-10150-03_GeoMx_DSP_Manu... 3.9MB including summaries here:

1) Slide Preparation

2) Bake slides

3) Deparaffinize and rehydrate FFPE tissue sections

4) Perform target retrieval

5) Expose RNA targets

6) Postfix: preserve tissue morphology for soft tissues

7) *In situ* hybridization

8) Perform stringent washes to remove off-target probes

At the point in which morphology markers are ready to be applied, follow steps below.

Quench autofluorescence

2   Dilute stock 40X True Black Plus aqueous (Biotium, catalogue #23014) to 1X in PBS.
Incubate sections in 1X True Black Plus for 10 min.
Note: any residual detergents may interfere with quenching efficacy

3   Remove quenching solution and wash sections thoroughly with cold PBS 5 times for 5 minutes each

Primary antibody stain

4   Stain sections in primary antibody solution diluted with GeoMx blocking buffer W
Antibody combinations consist of:
rabbit polyclonal anti-IBA1 (Fujifilm Wako, #019-19741, RRID:AB_839504)
     dilution: 1:500
rat IgG$_{2A}$monoclonal anti-galectin-3 clone eBioM3/38 preconjugated to eFluor 660
(Invitrogen, #50-5301-82, RRID:AB_11220276)
     dilution: 1:200
goat polyclonal anti-GFAP (Abcam, #ab53554, RRID:AB_880202)
     dilution: 1:2000
SYTO 83 Orange Fluorescent Nucleic Acid Stain (Invitrogen, #S11364)
     dilution: 5 µM

5   Remove antibody solution and wash sections thoroughly with cold PBS 3 times for 5 minutes each

Secondary antibody stain

6   Stain sections with donkey-host secondary antibodies conjugated to fluorophores AF488, AF594, or AF647 (Jackson Immunoresearch) for 2 hours at 25C in dark room environment.
Antibodies consist of:
       Donkey anti-rabbit IgG AF488 (1:250)
       Donkey anti-GFAP IgG AF594 (1:250)

7   Remove antibody solution Wash sections thoroughly with cold PBS 3 times for 5 minutes

GeoMx digital spatial profiling

8   Continue with procedure found in document

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Morphology marker segmentation strategy

9   After receiving the images created by GeoMx, set the following segmentation order:
1) IBA1+ , GFAP-, GAL3-
2) GAL3+, IBA1+, GFAP ignore
3) IBA1+, GAL3+, GFAP ignore

Protocol references

MAN-10150-03 GeoMx DSP Manual Slide Preparation
SEV-00087-05 GeoMx NGS DSP Instrument User Manual