



Development and Testing of IHC-Specific Antibodies at Cayman Chemical

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KEY FINDING GFAP and TMPRSS2 tissue localization was verified using in-house developed monoclonal antibodies.

INTRODUCTION

Immunohistochemistry (IHC) is a preferred application in clinical and academic research allowing for the specific detection for a target of interest using antibodies. Researchers can determine organ and cell type information in normal and disease state tissues, provided that high-quality specific antibodies are available. However, tools and reagents necessary for IHC testing can be costly and/or not accessible to some labs. Cayman Chemical has extensive experience with antigen and antibody development and has now optimized tissue sectioning and IHC testing on formalin-fixed, paraffin-embedded (FFPE) tissue blocks. Glial fibrillary acid protein (GFAP) is an intermediate filament protein, and elevated levels of citrullinated GFAP have been reported in the hippocampus of Alzheimer's patients. Antibodies to GFAP and citrullinated GFAP could reveal important data regarding levels of these proteins in Alzheimer's brain by IHC. TMPRSS2 is a transmembrane serine protease involved in the infection of viruses including SARS-CoV-2. TMPRSS2 localization by IHC could reveal information at sites of SARS-CoV-2 infection. Recombinant proteins were generated for human GFAP and TMPRSS2, which were used as antigens for antibody development in mice. Resulting monoclonal antibodies (mAbs) were tested by Western blot and IHC in human cerebral cortex and small intestine, respectively, due to documented GFAP and TMPRSS2 levels in these tissues.

ANTIGEN/ANTIBODY DEVELOPMENT

I Recombinant Protein Antigens

GFAP (human) (UniProt No. P14136)

- *E. coli* expressed (amino acids 2-432) – GST tag

TMPRSS2 (human) (UniProt No. O15393)

- *E. coli* expressed (amino acids 247-492) – 6XHis tag

II Mouse Immunization

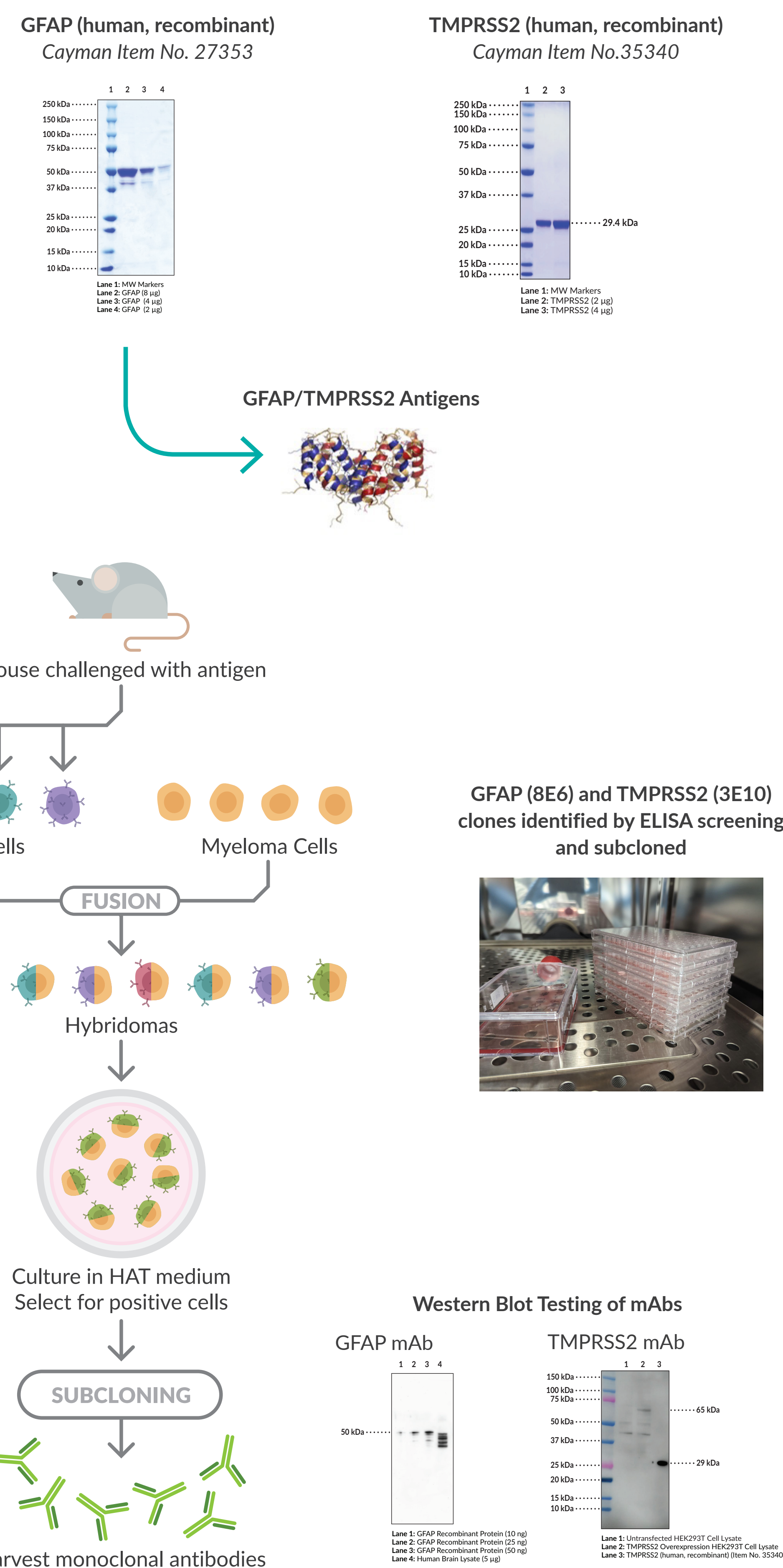
- GFAP/TMPRSS2 proteins emulsified with Freund's adjuvant and injected into mice (n=3)
- Response to the antigen checked twice by ELISA against the antigen
- Splenic fusion with best responding mouse

III Hybridoma Development (Screening/Subcloning)

- Potential hybridomas are screened by ELISA against the antigen
- Positive parental lines are subjected to two rounds of subcloning by limiting dilution to ensure clones of single cell origin
- Clones 8E6 (GFAP) and 3E10 (TMPRSS2) were isolated

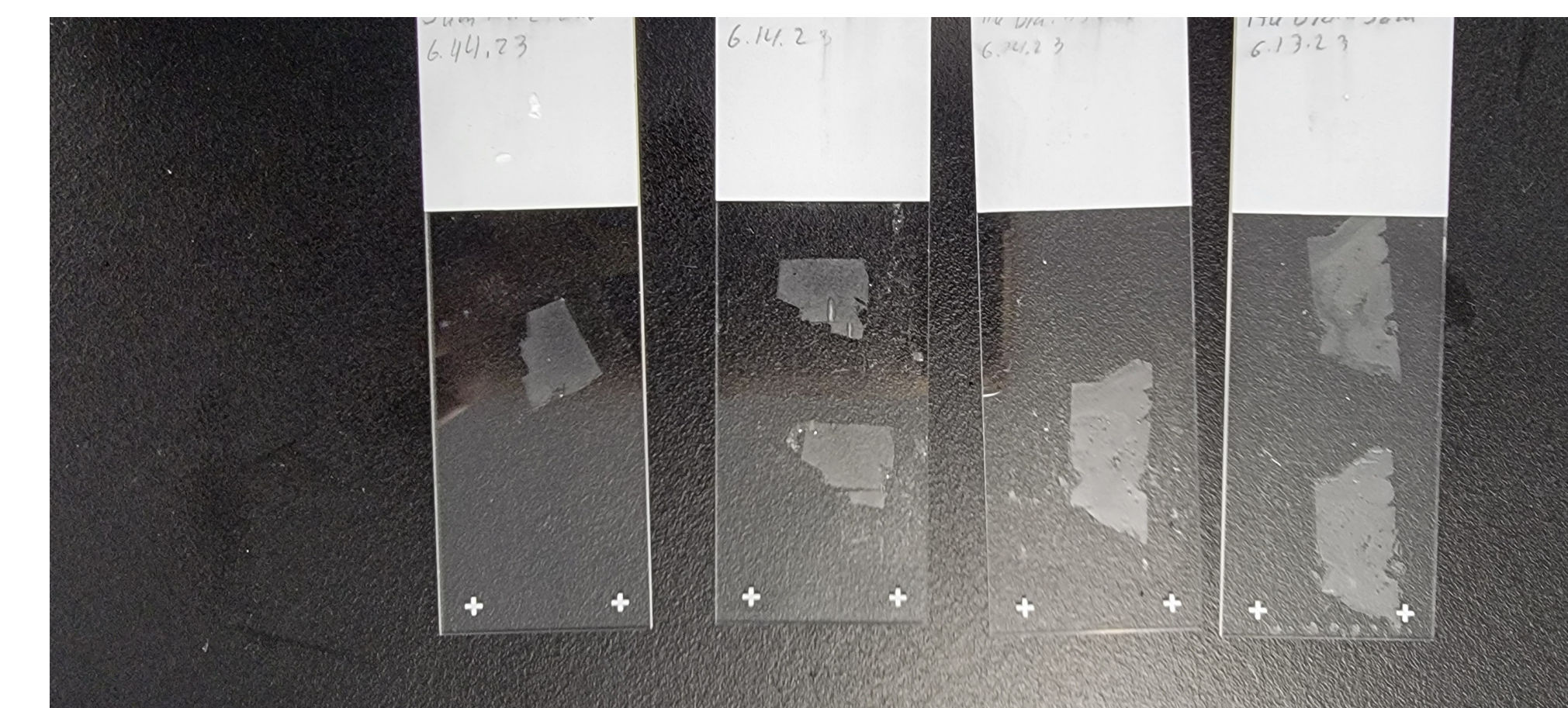
IV Purification and Antibody Testing

- Hybridomas for clones 8E6 (GFAP) and 3E10 (TMPRSS2) were grown, and supernatants were protein G purified and tested by Western blot prior to IHC



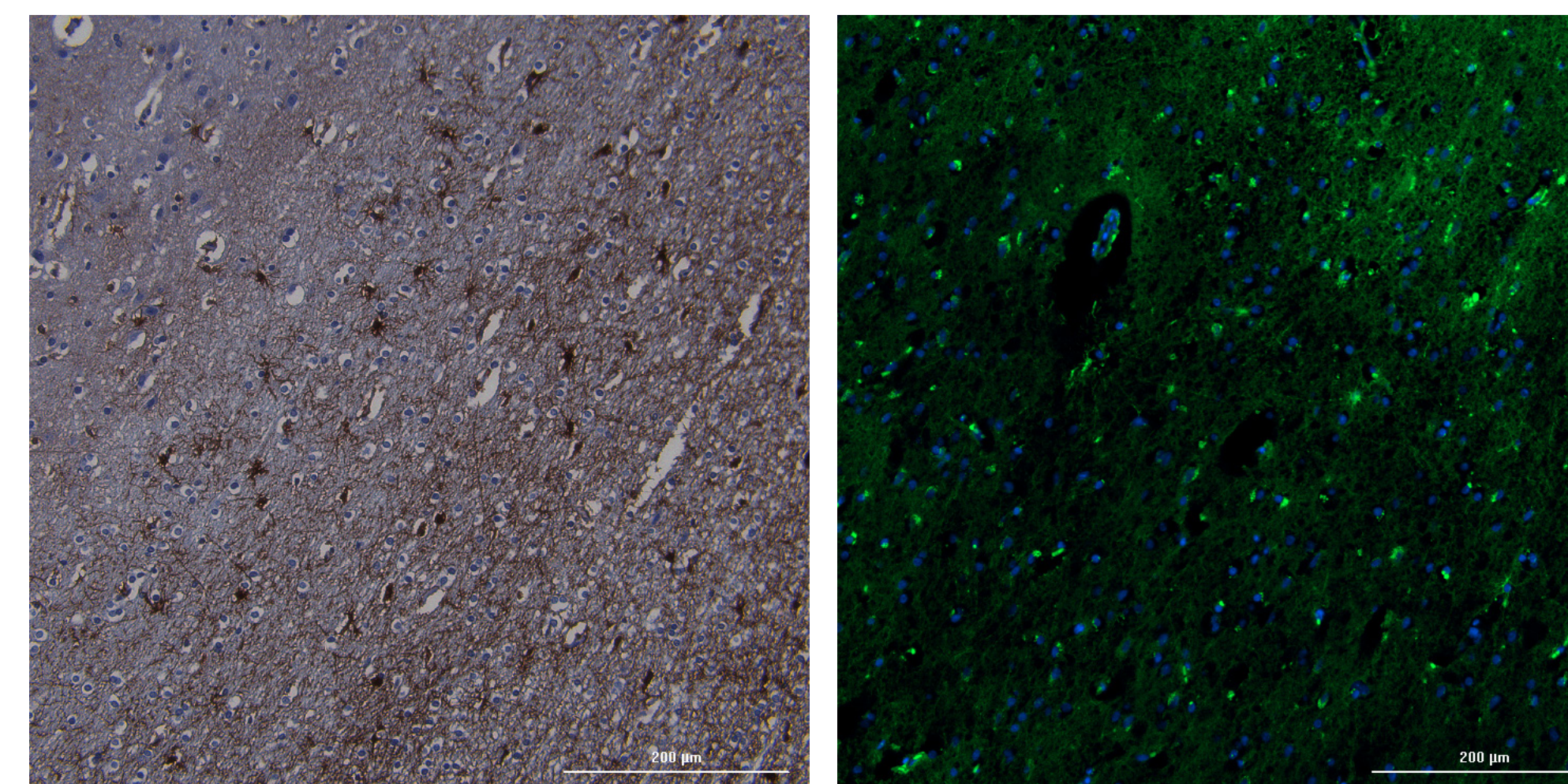
MICROTOMY AND SECTION STAINING

- Purified GFAP (clone 8E10) and TMPRSS2 (clone 3E10) mAbs
- FFPE blocks were sectioned by microtome for human small intestine and cerebral cortex (adult normal for both tissue types)
- Positively charged slides loaded with 5 μ m tissue sections
- For staining, slides are deparaffinized in xylenes and rehydrated
 - Antigen retrieval is conducted in 10 mM citric acid at 95°C for 30 minutes
 - Primary antibodies: 3-5 μ g/ml
 - Secondary antibody (HRP- or FITC-conjugated) is loaded at a 1:100 dilution. DAPI staining included for fluorescent imaging

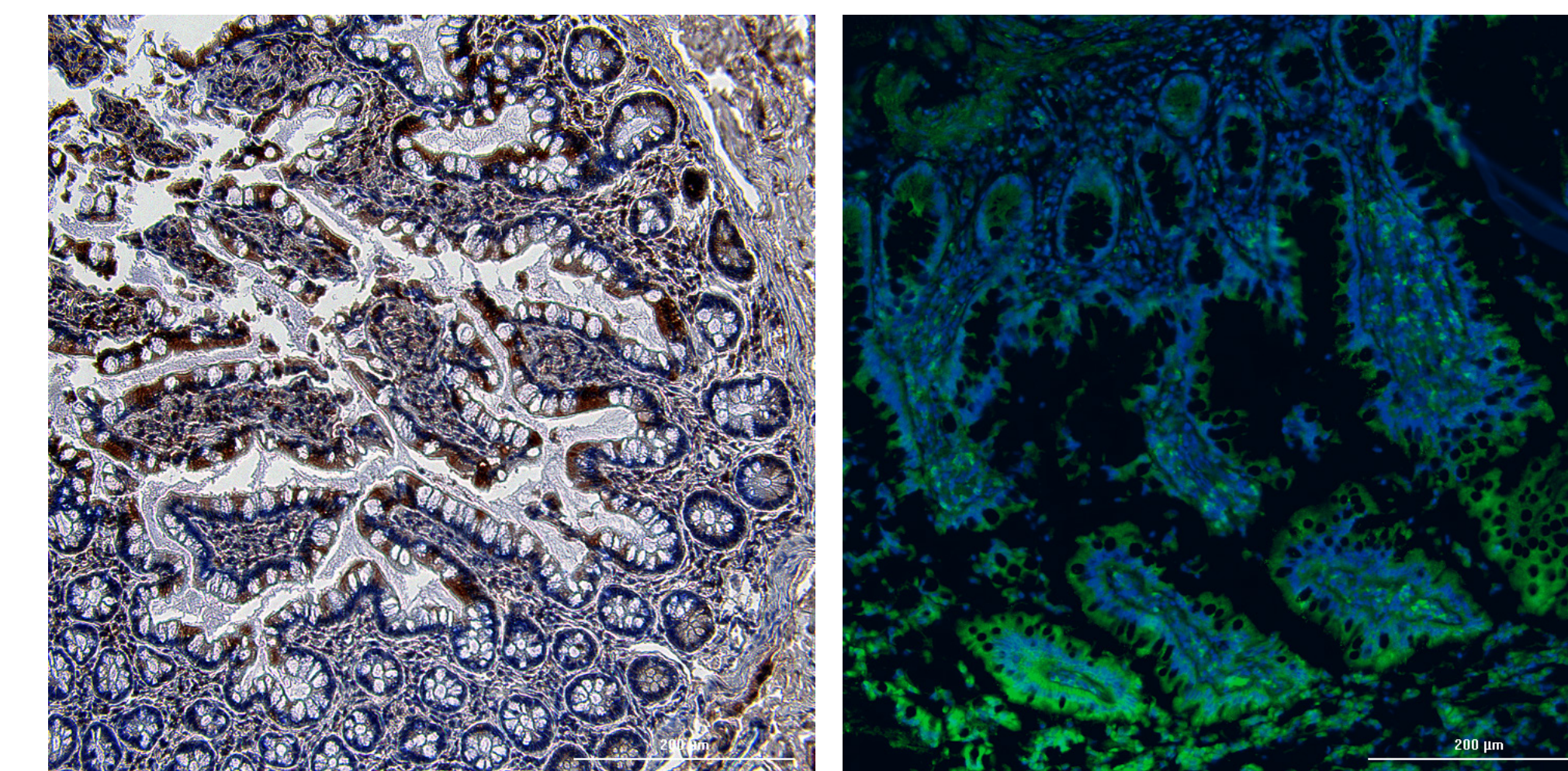


IHC IMAGING USING HRP AND FITC DETECTION

GFAP Monoclonal Antibody (Clone 3E6) - Human Cerebral Cortex - Cayman Item No. 28847



TMPRSS2 Monoclonal Antibody (Clone 3E10) - Human Small Intestine - Cayman Item No. 36357



SUMMARY

- Recombinant proteins were made for GFAP and TMPRSS2
- Protein antigens were immunized into mice for mAb development
- GFAP (8E6) and TMPRSS2 (3E10) clones were isolated
- Human cerebral cortex and small intestine FFPE blocks were sectioned (5 μ m) and mounted
- GFAP and TMPRSS2 mAbs were tested on tissue and imaged with detection observed comparable to reported protein localization
- GFAP localized to cerebral astrocytes and TMPRSS2 localized to small intestine epithelium

Future Testing

- GFAP and citrullinated GFAP mAb comparison in Alzheimer's brain tissue sections
- TMPRSS2 mAb IHC in lung tissue, which is the site of SARS-CoV-2 infection, using FFPE sections

Cayman Chemical Antibody Services Available

- Full antigen design and synthesis (peptide, protein, small molecule)
- Antigen conjugation and immunization (pAb and mAb)
- FFPE sectioning and IHC optimization
- Wide range of application-specific Ab testing
- Recombinant antibody development



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