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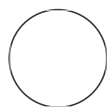
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Immunohistochemistry of tissue sections from formalin-fixed paraffin embedded (FFPE) samples V.1

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DISCLAIMER

The protocol was developed and performed by Terri Li and Can Gong at the University of Chicago Human Tissue Resource Center core facility

ABSTRACT

H&E staining and immunohistochemistry of tissue sections from formalin-fixed paraffin embedded (FFPE) murine tissue samples were performed by the University of Chicago Human Tissue Resource Center core facility.

Biotinylated anti-mouse Tn IgM antibody (clone 5F4) and WE scFv staining

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- 1.1 Tissue sections were deparaffinized and rehydrated through xylenes and serial dilutions of ethanol to deionized water.
- 1.2 Tissue sections were incubated in antigen retrieval buffer (DAKO S2367) and heated in a steamer at over 97°C for 20 minutes.
- 1.3 Biotinylated anti-Tn antibody (1:20) or biotinylated WE scFv (1:200) were applied to tissue sections for 1 hour at room temperature.
- 1.4 After washing, the biotinylated reagent was detected with the Elite kit (Vector Laboratories PK-6100) and DAB (DAKO K3468) system.

2 Anti-CD3 (Abcam ab135372 clone SP162), anti-F4/80 (BioRad MCA497GA clone A3-1), anti-Ly6G (BioLegend 127602 clone 1A8) and anti-cleaved Caspase 3 (Cell Signaling Technology 9661) staining

- 2.1 The slide was stained using Leica Bond RX automated stainer.
- 2.2 After deparaffinization and rehydration, tissue section was heat treated for 20 minutes with antigen retrieval solution (Leica Biosystems AR9961).
- 2.3 Respective antibody (1:100) was applied on tissue sections for 60 minutes at room temperature and the antigen-antibody binding was detected with Bond Polymer Refine Detection (Leica Biosystems DS9800) without post-primary reagent.

- 3 Tissue sections were stained with hematoxylin for counterstaining and were covered with cover glasses.