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

In 2 collections

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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.