PD-L1 22C3 LDT on Dako automate

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Abstract

PD-L1 LDT on Dako Autostainer Link 48 (ASL48) using the concentrate 22C3 antibody. This protocol was developed in the laboratory of Dr. Paul Hofman and Dr Marius Ilie (Pasteur Hospital, Nice, France).

For the purpose of protocol development 95% of clinical specimens consisted of surgical resection specimens and the remaining 5% were bronchial biopsies and ransbronchial mediastinal lymph node biopsies.

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Protocol

Step 1.
The specimens are sectioned at a thickness of 3-µm and stained on glass slides stored at 4°C within 3 days after sectioning.

Step 2.
Deparaffinization, rehydration and antigen retrieval is performed on PT Link (Dako PT100) using the EnVision™ FLEX Target Retrieval Solution, low pH 6.0 for 53 minutes.

Step 3.
Following FLEX peroxidase block for 5 minutes, specimens are incubated with primary mouse monoclonal antibody anti-PD-L1 (Ref. M365329, Dako) using a concentration of 1:50 for 60 minutes at room temperature.

Step 4.
Specimens are then incubated with the EnVision™ FLEX+ Mouse LINKER for 30 minutes at room temperature, followed by incubation with the EnVision™ FLEX HRP visualization reagent for 30 minutes at room temperature.

Step 5.
The enzymatic conversion of the subsequently added 3,3’-diaminobenzidine tetrahydrochloride (DAB) chromogen followed by DAB enhancer resulted in precipitation of a visible reaction product at the site of antigen. The specimens are then counterstained with Hematoxylin and coverslipped.

Step 6.
Each IHC run contained a positive control (on-slide tonsil tissue) and a negative antibody control (buffer, no primary antibody).

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