PD-L1 22C3 LDT on VENTANA BenchMark ULTRA automate

Marius Ilie,Paul Hofman

Abstract

Optimised VENTANA BenchMark ULTRA protocol for PD-L1 assay using the concentrate 22C3 antibody.

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

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Guidelines

Scoring and Interpretation of the staining:
The Tumor Proportion Score (TPS) of the PDL-1 expression for the specimen

The TPS is the percentage of viable cells showing partial or complete staining (≥ 1%) relative to all viable tumor cells positive or negative present in the sample. TPS should be evaluated on at least 100 viable cells in the specimen.
Specimens are interpreted as having:
- No PDL-1 expression: TPS < 1%
- Any PDL-1 expression: TPS ≥ 1%
Specimens expressing PDL-1 can be divided into PDL-1 expressing: TPS 1% to 49% and high PD-L1 expressing TPS≥ 50%.
For more information regarding the Interpretation of the scoring please refer to PDL-1 IHC 22C3 PharmDx-Interpretation manual:
http://www.dako.com/us/29109_pd-l1-ihc-22c3-interpretation-manual.pdf

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 by CC1 (prediluted; pH 8.0) antigen

retrieval solution (Ventana Medical Systems, Roche Group, Tucson, AZ, USA), performed on the BenchMark ULTRA automated slide stainer (Ventana) for 64 minutes at 100°C (default temperature on ULTRA).

Step 3.
The 22C3 assay was developed for use on the BenchMark ULTRA automated staining platform (Ventana) in combination with primary mouse monoclonal antibody anti-PD-L1 (Ref. M365329, Dako) using a concentration of 1:50 for 32 minutes at 37°C, followed by visualization with the OptiView DAB IHC Detection Kit (Ventana) and OptiView Amplification Kit (Ventana) for 12 minutes.

Step 4.
The Ventana Antibody Diluent (catalog number 251-018) should be used to dilute the primary antibody anti-PD-L1 for use on BenchMark ULTRA.

Step 5.
The specimens are then counterstained with Hematoxylin II and Bluing Reagent (Ventana) and coverslipped. Each IHC run contains a positive control (on-slide tonsil tissue) and a negative antibody control (buffer, no primary antibody).

ANNOTATIONS

Gabriela Bucur 18 Aug 2017

Dear Dr. Marius Ilie,

I would like to inform you that my name is Gabriela Bucur and I represent a medical genetic clinic - Personal Genetics Bucharest. We have also a department of Pathology and we intend to perform PDL1 clone 22C3 DAKO on Ventana in our lab.

Please help me with an information on controls. The negative antibody control reffers to the fact that should be done one negative control per run or for every patient slide should be performed an additional slide?

I understand that a buffer is used instead primary antibody. Please let me what buffer should be used.

I am asking that because trying to clarify myself, I saw in Autostainer Link 48 PDL1 22C3 pharmDx code SK006 protocol that for every patient sample must be done an additional slide which is treated with Negative Reagent Control (which is delivered in PDL1 22C3 pharmDx kit).

Thank you very much.

Yours faithfully,

Gabriela Bucur

Personal Genetics

Bucharest