

PDL-1 Targeted therapy demands

IMUNOHISTOCHEMISTRY PROTOCOL

A tumour is a very complex and narcissist structure. It lives on itself by growing and raising adequate environment to follow its natural malignant dissemination. The host has to be eager enough to get rid of it. Sometimes or often, it is impossible without external help through correct targeted medication, in advanced staged tumours.

The medication might be directed to the malignant proliferating cells and/or to help the host immune system to control that proliferation capacity. The first group of drugs goes from conventional chemotherapy till actual molecular pathology determined targets directed therapy and the second, has been explored in melanoma, lung cancer... by determining if the tumour cells are indeed engaged in blocking host – T cells or other immune cells.

Anatomical Pathology and Molecular Pathology Labs contribute to therapy selection after tumour characterization either by using DNA/RNA and by submitting tumour sections to immunohistochemical determinations. Pathologists, Technicians and Bio-Technicians develop their running after tumour biology knowledge through the available robust and certified equipment.

As a primary immunosuppressive tumour driver, **PD-L1** – Programmed of Death Ligand 1 – overexpression may be an important facilitator for tumour growth and metastasis. PD-L1 has been detected in up to 50% of human cancers, making the PD-L1 pathway a focus of cancer research and directed therapy.

For this reason, the **Institute of Anatomical and Molecular Pathology of the Faculty of Medicine of Coimbra**, has been active in the optimization of PD-L1 biomarker. In this way, the protocol validated by the IAP-PM was designed through the Leica Bond TM system.

The reagents used correspond to Bond Polymer Refine Detection (catalog no. DS9800), Leica Microsystems [™], and includes the following steps for the corresponding actions:

Peroxide blocking, Marker, Post primary antibody, Polymer liaison, Mix DAB Refine Staining, Bond DAB Enhancer, if it's your choice, and Haematoxylin counter stain. Before the blocking pass, pre-treatment maybe useful.

This technical sequence is applicable to other immunostaining stations.

We suggest positive controls to be used in the calibration of the protocol (pulmonary macrophages, mature placenta... basal cells counterstaining).

This protocol is easily reproduced for the PD-L1 DAKO 22C3 antibody.

For targeted therapy purpose, we advise the following *cut-off*:

Negative – 0% / + - < 5% / ++ - 5- 50% / +++ - > 50%.

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