



PathAI PD-L1 (28-8) Bundle on navify® Digital Pathology

REF 2012300001

User Manual

RUO





NOTE: The PathAI PD-L1 (28-8) Bundle on **navify** Digital Pathology is intended for research use only. Not for use in diagnostic procedures. Access to the product is intended for authorized users only. Contact your institution for access approval.

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Chapter 1: Introduction

This chapter includes:

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Product Description

The PathAI PD-L1 (28-8) Bundle is a software tool meant to identify and enumerate cells within tissue stained with a PD-L1 (28-8) staining assay and is intended for research use only. Not for use in diagnostic procedures:

Algorithm	Manufacturer
PathAI PD-L1 (28-8) Melanoma RUO	The PathAI PD-L1 (28-8) Melanoma algorithm is a software tool intended for identification and quantification of tumor cells, immune cells, and tissue regions within whole slide images (WSI) of formalin-fixed, paraffin-embedded (FFPE) melanoma tissue stained with PD-L1 (28-8 clone) and scanned on a VENTANA DP 200 whole slide scanner. PathAI PD-L1 (28-8) Melanoma algorithm is intended for Research Use Only. Not for use in diagnostic procedures.
PathAI PD-L1 (28-8) Urothelial Carcinoma RUO	The PathAI PD-L1 (28-8) Urothelial Carcinoma algorithm is a software tool intended for identification and quantification of tumor cells, immune cells, and tissue regions within whole slide images (WSI) of formalin-fixed, paraffin-embedded (FFPE) Urothelial Carcinoma (UC) tissue stained with PD-L1 (28-8 clone) and scanned on a VENTANA DP 200 whole slide scanner. PathAI PD-L1 (28-8) Urothelial Carcinoma algorithm is intended for Research Use Only. Not for use in diagnostic procedures.

Algorithm	Manufacturer
PathAI PD-L1 (28-8) NSCLC RUO	The PathAI PD-L1 (28-8) NSCLC algorithm is a software tool intended for identification and quantification of tumor cells, immune cells, and tissue regions within whole slide images (WSI) of formalin-fixed, paraffin-embedded (FFPE) Non-Small Cell Lung Cancer (NSCLC) tissue stained with PD-L1 (28-8 clone) and scanned on a VENTANA DP 200 whole slide scanner. PathAI PD-L1 (28-8) NSCLC algorithm is intended for Research Use Only. Not for use in diagnostic procedures.
PathAI PD-L1 (28-8) HNSCC RUO	The PathAI PD-L1 (28-8) HNSCC algorithm is a software tool intended for identification and quantification of tumor cells, immune cells, and tissue regions within whole slide images (WSI) of formalin-fixed, paraffin-embedded (FFPE) Head and Neck Squamous Cell Carcinoma (HNSCC) tissue stained with PD-L1 (28-8 clone) and scanned on a VENTANA DP 200 whole slide scanner. PathAI PD-L1 (28-8) HNSCC algorithm is intended for Research Use Only. Not for use in diagnostic procedures.

Supported Software

The PathAI PD-L1 (28-8) Bundle runs on Roche **navify**® Digital Pathology, version 2.2. This PathAI user manual applies to each algorithm version in the PathAI PD-L1 (28-8) Bundle:

- PathAI PD-L1 (28-8) HNSCC RUO (v1.0.x and later)
- PathAI PD-L1 (28-8) Melanoma RUO (v1.0.x and later)
- PathAI PD-L1 (28-8) NSCLC RUO (v1.0.x and later)
- PathAI PD-L1 (28-8) Urothelial Carcinoma RUO (v1.0.x and later)

About This Manual

The manual provides details about the slide and scanner requirements, image analysis workflow, and the image analysis results generated by the PathAI PD-L1 (28-8) Bundle. For instructions on running the IHC using the Dako Autostainer Link 48, scanning slides using the VENTANA DP 200 scanners, or using the features and supported workflows in the **navify** Digital Pathology, see their respective user manuals.

Symbol Definition

The following table defines symbols that appear in this user manual:

Symbol	Definition
	Indicates the product is <i>"For research use only. Not for use in diagnostic procedures."</i>
	Indicates the manufacturer's catalog number so the product can be identified.
	Indicates the need for the user to consult the instructions for use.
	Indicates the manufacturer of the product.

Product Names

The following product names are referenced throughout this user manual:

Product Names	Manufacturer
PathAI PD-L1 (28-8) Bundle	PathAI, Inc.
navify Digital Pathology	Roche Diagnostics
VENTANA DP 200 slide scanner	Roche Diagnostics
Dako Autostainer Link 48	Agilent
PD-L1 IHC 28-8 pharmDx assay kit	Agilent
EnVision FLEX visualization system	Agilent

About Algorithm Limitations

The PathAI PD-L1 (28-8) Bundle analyzes tissue in whole slide images stained with the PD-L1 IHC (28-8) pharmDx assays.

Guidelines for Ensuring Image Quality

To improve image quality, use the following guidelines:

- For each staining run, follow the manufacturer's recommendations for each applicable assay using all the positive and negative quality control materials.
- Validate the applicable assay staining run by reviewing the PD-L1 control slides manually using conventional light microscopy to ensure the stain quality of the slide before generating a whole slide image with the slide scanner.
- If you determine the quality of the control slides are not acceptable during your manual examination, re-stain the slide and review the quality again using conventional light microscopy. For recommendations, see the manufacturer's assay documentation.
- For the PD-L1 IHC 28-8 pharmDx assay, follow the manufacturer's interpretation guidelines to help you evaluate stain quality.

Guidelines for Understanding Algorithm Limitations

When reviewing algorithm results, note the following algorithm limitations:

- The algorithm results are only as good as the quality of the immunohistochemistry (IHC) and the quality of the subsequent whole slide image that is analyzed.
- The algorithm may generate incorrect scores if the slide was improperly scanned or has:
 - Significant artifacts obscuring cancer cells; or
 - Abnormal staining.
- The algorithm may misidentify cells due to the presence of several staining factors:
 - Weak cytoplasmic/membranous staining;
 - Strong immune cell staining overlapping with tumor cell staining where there is significant intermixed inflammation;
 - Strong PD-L1 staining obscuring cell identity;
 - Tumor cells with cytoplasmic blush; and
 - Non-target staining.

These factors may result in the algorithm misidentifying tumor cells as non-tumor cells and non-tumor cells as positive tumor cells.

Contacting Roche Support

If you have questions pertaining to the appropriate scanning of images (VENTANA DP 200 slide scanner), or effective utilization of **navify** Digital Pathology, contact your local affiliate or Roche Service representative and reference the following product information:

- *PathAI PDL1 (28-8) Algo Bundle Ult'd Sub*
- *Roche product number 0909842519001*

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Chapter 2: Product Overview

This chapter includes:

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About the Algorithm Test Principles

The PathAI PD-L1 (28-8) Bundle uses derived overlays to visualize classification of tissue stained with PD-L1 (28-8) pharmDx as *Cancer*, *Stroma*, *Necrosis*, and *Artifact*. (*Figure 1*).

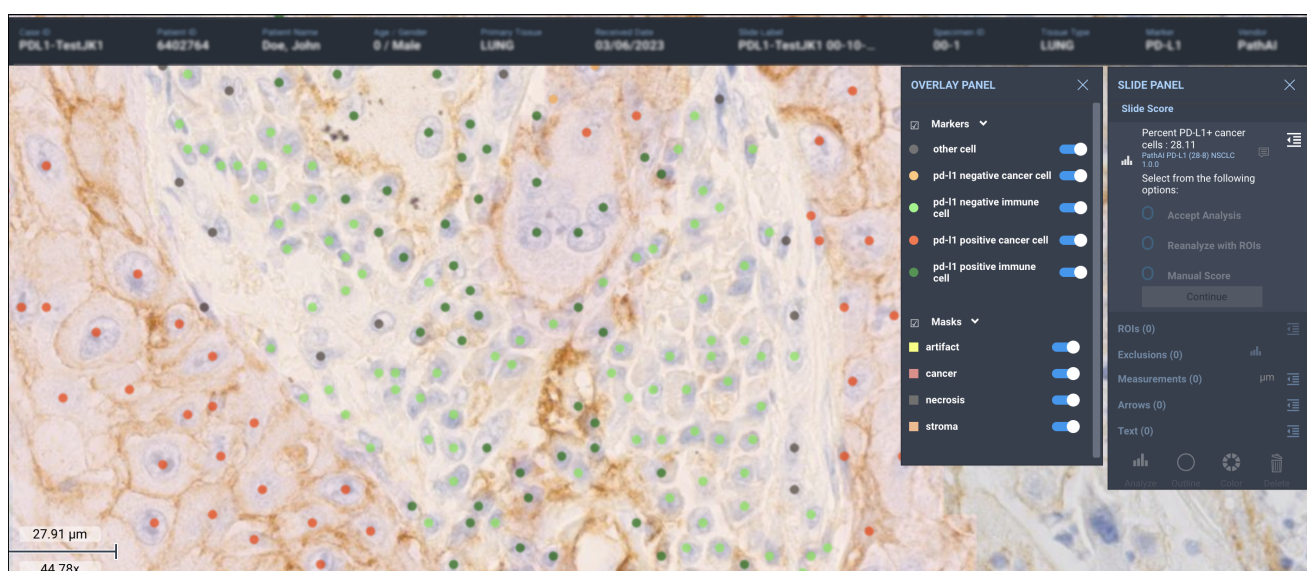


Figure 1 navify Digital Pathology showing PD-L1 28-8 overlays

Steps in Image Analysis

This process happens in the background.

1. Split image into tiles for processing by the convolutional neural network.
2. Classify tissue regions as one of the following tissue types:
 - Cancer
 - Cancer Stroma
 - Necrosis
 - Artifact
3. Classify cells as one of the following cell types:
 - PD-L1+ Cancer Cell
 - PD-L1 Negative Cancer Cell
 - PD-L1+ Immune Cell
 - PD-L1 Negative Immune Cell
 - Other Cell
4. Calculate the slide-level results which summarize the number and proportions of different cell types within different tissue regions.

Cell Identification and Scoring

Cell locations and classifications are identified by parsing overlays generated by the deep learning network. The classification for any individual cell is based on the cell type with the highest probability as assessed by the deep learning network.

How the Algorithm Reports Scores

The PathAI PD-L1 (28-8) Bundle reports each score to one digit in the tenths place (for example, 4.8%).

Chapter 3: Algorithm Workflow

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About the Algorithm Workflow

The workflow for the PathAI PD-L1 (28-8) Bundle includes the following procedures:

- Staining the tissue on the slide using the PD-L1 IHC 28-8 pharmDx assay kit with the Dako Auto-stainer Link 48.
- Scanning the slide with the VENTANA DP 200 slide scanner.
- Analyzing and scoring the whole slide image with the PathAI PD-L1 (28-8) Bundle.

Antibody Staining

The PD-L1 IHC 28-8 pharmDx antibody identifies PD-L1+ cells in tissue:

- Follow the instructions provided in the indication-specific PD-L1 IHC 28-8 pharmDx manual for tissue preparation, staining, and review of controls.
- Restain the slides if staining does not meet the guidelines outlined in the PD-L1 IHC 28-8 pharmDx manual.

Slide and Scanning Criteria

The PathAI PD-L1 (28-8) Bundle accepts the following whole slide image (WSI) and scanner types as input per accession:

Types	Description
Slides	• 1 PD-L1 (28-8) stained slide (required) • 1 Negative Control Reagent (NCR) stained slide (optional) • 1 H&E stained slide (optional)
Image	• BIF(F) • TIF(F)
Scanner	• VENTANA DP 200 slide scanner* 20x, 40x magnification

**For scanning instructions, see the VENTANA DP 200 Slide Scanner User Guide.*

Creating and Analyzing Slide Images

To perform this task, complete the following steps:

1. Use a Dako Autostainer Link 48 with the EnVision FLEX visualization system to stain the tissue sample with the PD-L1 28-8 antibody.
2. Scan the slide with the VENTANA DP 200 slide scanner in BIF(F) or TIF(F) file format at 20X or 40x magnification and at one z-plane.

The VENTANA DP 200 slide scanner transfers the slide image to the image management system.

3. Create a case in **navify** Digital Pathology either manually in **Data Management** or automatically through communication with the laboratory information system. For instructions, see the *Roche navify Digital Pathology User Guide*.

Chapter 4: Algorithm Results Overview

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Viewing Colored Overlays

After an algorithm run, the software produces colored overlays on the analyzed slide image in the **VIEWER**. You can view the definitions of each color and toggle them on and off from the **OVERLAY PANEL** (*Figure 2*).

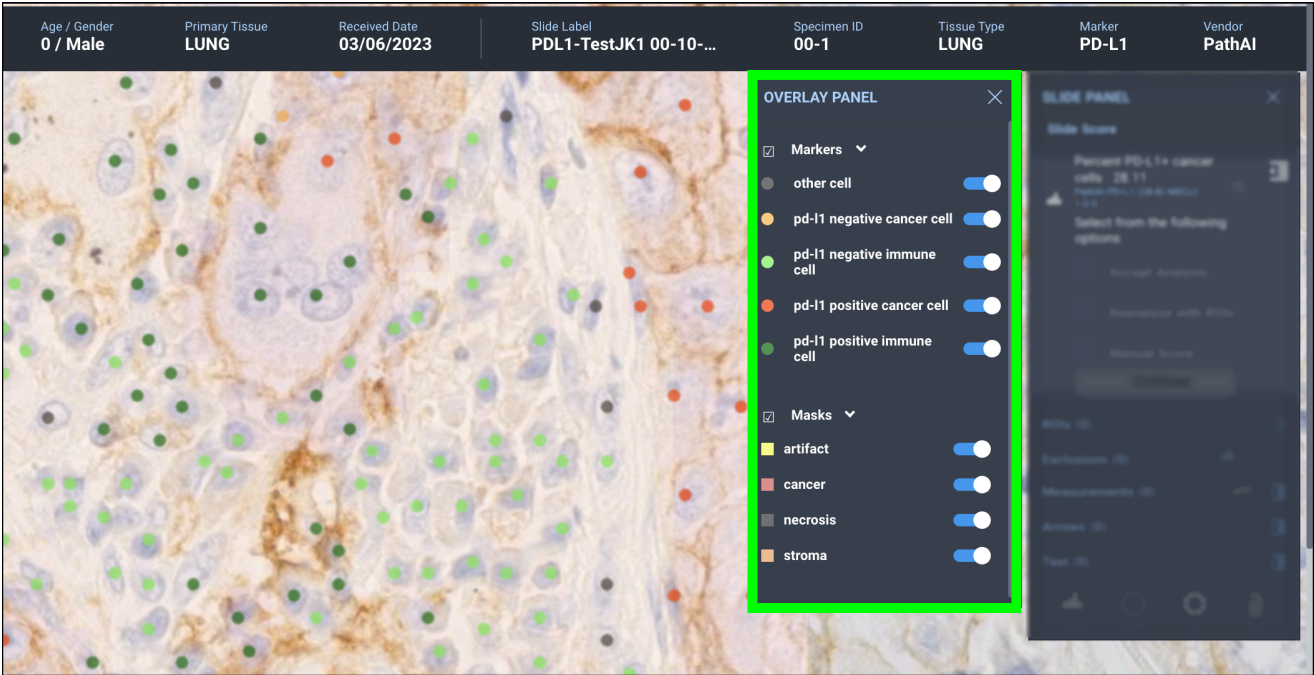


Figure 2 navify Digital Pathology OVERLAY PANEL

Color Definitions in Overlays

The colored overlays that appear on analyzed slides highlight specific **cells** and **tissue** regions detected by the algorithm. Cells are marked by colored squares in the whole slide image, while tissue regions are marked by colored overlays. The following tables define each color:

Cells > Cell Overlays

Group	Color	Definition
Cancer Cells	 Red	PD-L1 Positive Cancer Cell
	 Orange	PD-L1 Negative Cancer Cell
Immune Cells	 Dark green	PD-L1 Positive Immune Cell
	 Light green	PD-L1 Negative Immune Cell

Tissue Regions > Tissue Overlays

Group	Color	Definition
Evaluable Tissue	 Dark Red	Cancer
	 Dark Orange	Stroma
	 Black	Necrosis
Slide Quality	 Yellow	Artifact

Viewing Quantitative Scoring Results

After the algorithm run, you can view the quantitative scoring results in the **VIEWER's** slide panel. To open this panel, click **VIEWER** and select **Slide Panel** (Figure 3).



Figure 3 navify Digital Pathology SLIDE PANEL

Results	Description
Percent PD-L1+ cancer cells	Number of detected PD-L1+ cancer cells in tumor divided by number of detected cancer cells in tumor, expressed as a percentage (rounded to nearest hundredth).
Number of immune cells in tumor	Number of immune cells detected in evaluable tumor tissue.
Percent PD-L1+ immune cells in tumor	Number of detected PD-L1+ immune cells in tumor divided by number of immune cells in tumor, expressed as a percentage (rounded to nearest hundredth).
Total number of PD-L1+ cancer cells	Total number of PD-L1+ cancer cells detected in evaluable tissue.
Number of PD-L1+ cancer cells in cancer epithelium region.	Total number of PD-L1+ cancer cells detected in Cancer overlay region.

Results	Description
Proportion of PD-L1+ cells to all cancer cells	Number of detected PD-L1+ cells (both tumor and immune) in tumor divided by number of detected cells in tumor, multiplied by 100 and capped at 100 (rounded to nearest whole number).
Total number of cancer cells	Total number of cancer cells detected in evaluable tissue.
Number of PD-L1+ immune cells in tumor	Number of PD-L1+ immune cells detected in evaluable tumor tissue.
Total number of nucleated cells	Total number of nucleated cells (tumor and immune cells) in evaluable tissue.
Number of PD-L1+ immune cells in cancer epithelium region	Number of PD-L1+ immune cells detected in the Cancer overlay region.
Number of PD-L1+ immune cells in cancer stroma region	Number of PD-L1+ immune cells detected in the Cancer Stroma overlay region.
Evaluable tissue area (mm ²)	Area within the whole slide image of evaluable tissue.
Artifact detected (%)	Percent of evaluable tissue area with artifact detected.
Tumor area detected (%)	Percent of evaluable tissue area with tumor detected.

Chapter 5: Generating Reports

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About Generating Reports

After an algorithm run, you can generate reports containing summary details about each case. The type of data included in the report is based on templates configured by the administrator. For details, see Producing and signing out a report in the *Roche navify Digital Pathology User Guide*.

Understanding the Report

The report includes a tabular summary of all the quantitative scoring results described in [Chapter 4](#).

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Chapter 6: Troubleshooting

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Re-triggering a Failed Analysis

You must have Administrator privileges to perform this troubleshooting task.

If an error occurs during the whole slide analysis, an error message appears under the slide. To address the failure, perform the following steps:

1. Login to **navify** Digital Pathology.
2. Navigate to **Administer Settings** and choose **Job Queue**.



NOTE: The administrator settings is only available if you have administrator privileges.

3. Choose the **Failed** tab.
All slides with failed analysis are displayed.
4. Choose the slide to be analyzed then choose the **Start Job** button.
The slide moves to **Canceled/Completed** tab when done.

Best Practices for Excluding Areas from Analysis

To ensure the algorithm is analyzing the tumor region appropriately, perform the following steps:

1. Turn off the algorithm overlays on the PD-L1 slide to identify the tumor area, consulting the matched H&E-stained slide as necessary.
2. Evaluate whether the algorithm is over-calling the tumor region. For example, determine if the algorithm overlay labels non-tumor tissue incorrectly as *cancer* or *cancer stroma*. Specifically:
 - a. Do the combined overlays for *cancer* and *cancer stroma* overlie non-tumor regions?
 - b. If so, draw exclusion ROI annotations to remove these overcalled tumor regions from the algorithm analysis.
3. If this mitigation does not address the problem, reject the finding and manually score the case.



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