

# PhenoCode Discovery Immune Activation and Proliferation Human Protein Module

## PRODUCT INFORMATION

See Page 2 for detailed information.

### STORAGE

- Antibodies: 4°C
- Reporters: -20°C\*

\*See PhenoCycler-Fusion User Guide (Doc# PD-000011) for details.

### STABILITY

See expiration date of each antibody and reporter tube.

### ANTIGEN RETRIEVAL

AR9 (Akoya, Part# AR900250ML)

### SPECIES REACTIVITY

Human

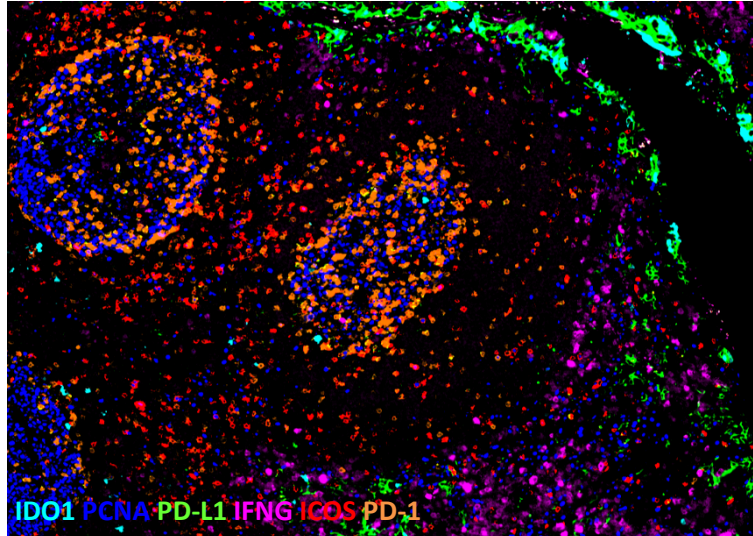
### TISSUE TYPE

FFPE

### SYSTEM COMPATIBILITY

The panel module has been optimized for the PhenoCycler-Fusion system.

Protocol for tissue staining can be found in the PhenoCycler-Fusion User Guide (Doc# PD-000011).



Human FFPE tonsil tissue section was stained with the PhenoCode Discovery Immune Activation and Proliferation Human Protein Module and imaged on the PhenoCycler-Fusion system. Antigen retrieval was performed using AR9 (Akoya, Part# AR900250ML). All antibodies were diluted 1:200.

The PhenoCode™ Discovery Immune Activation and Proliferation Human Protein Module enables detection of 6 markers on multiple tissues using the PhenoCycler®-Fusion system. This module is intended to help researchers characterize immune escape and activation mechanisms within the tumor, and to identify which checkpoint inhibitors could be used for its treatment. It has been tested on tonsil and lung cancer tissues.

| Target | Biological Relevance              |
|--------|-----------------------------------|
| PD-1   | Checkpoint receptor               |
| PD-L1  | Checkpoint ligand                 |
| ICOS   | Checkpoint receptor               |
| PCNA   | G1 and S phase marker             |
| IDO1   | Multifunctional/Immune inhibitory |
| IFNG   | Activated lymphocytes             |

## PhenoCode Discovery Immune Activation and Proliferation Human Protein Module

### Contents of PhenoCode Discovery Immune Profiling Human Protein Core

The PhenoCode Discovery Immune Activation and Proliferation Human Protein Module contains the following conjugated antibodies and reporters. Recommended starting dilutions are listed for staining tonsil and lung cancer tissue on the PhenoCycler-Fusion system. Further optimization may be needed depending on the tissue.

| Target | Catalog                 | Clone ID | Barcode | Reporter               | Dilution Tonsil | Dilution Cancer |
|--------|-------------------------|----------|---------|------------------------|-----------------|-----------------|
| PD-1   | <a href="#">4550038</a> | AKYP0070 | BX046   | Alexa Fluor™ 647-RX046 | 1:200           | 1:100           |
| PD-L1  | <a href="#">4550128</a> | AKYP0103 | BX067   | Alexa Fluor™ 647-RX067 | 1:200           | 1:100           |
| ICOS   | <a href="#">4550129</a> | AKYP0090 | BX065   | Alexa Fluor™ 647-RX065 | 1:200           | 1:200           |
| PCNA   | <a href="#">4450097</a> | AKYP0085 | BX036   | Alexa Fluor™ 750-RX036 | 1:200           | 1:200           |
| IDO1   | <a href="#">4450098</a> | AKYP0084 | BX027   | Alexa Fluor™ 750-RX027 | 1:200           | 1:200           |
| IFNG   | <a href="#">4250062</a> | AKYP0093 | BX020   | Atto 550-RX020         | 1:200           | 1:200           |

### Cycle Configuration on PhenoCycler-Fusion

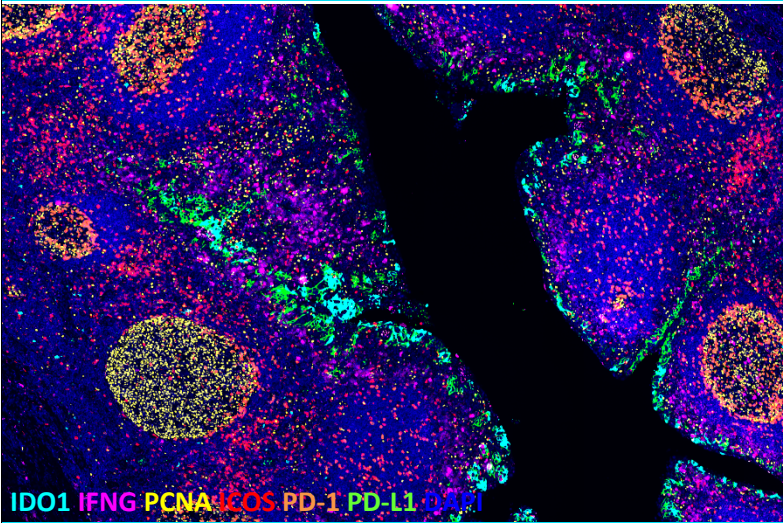
The PhenoCode Discovery Immune Activation and Proliferation Human Protein Module was run using the following run cycle configuration on the PhenoCycler-Fusion system using standard recommendations for nuclear stain and blank cycles. The order and cycle configuration of markers can be changed as needed. Recommended exposure times are listed for imaging tonsil and lung cancer tissue. Exposure times are for PhenoCycler-Fusion only.

| TONSIL TISSUE |                |                    |                 |                    |                 |                    |
|---------------|----------------|--------------------|-----------------|--------------------|-----------------|--------------------|
| Cycle Order   | Atto 550       |                    | Alexa Fluor 647 |                    | Alexa Fluor 750 |                    |
|               | Target-barcode | Exposure Time (ms) | Target-barcode  | Exposure Time (ms) | Target-barcode  | Exposure Time (ms) |
| 1             | IFNG-BX020     | 125                | PD-1-BX046      | 125                | --              | --                 |
| 2             | --             | --                 | PD-L1-BX067     | 125                | IDO1-BX027      | 125                |
| 3             | --             | --                 | ICOS-BX065      | 125                | PCNA-BX036      | 80                 |

| CANCER TISSUE |                |                    |                 |                    |                 |                    |
|---------------|----------------|--------------------|-----------------|--------------------|-----------------|--------------------|
| Cycle Order   | Atto 550       |                    | Alexa Fluor 647 |                    | Alexa Fluor 750 |                    |
|               | Target-barcode | Exposure Time (ms) | Target-barcode  | Exposure Time (ms) | Target-barcode  | Exposure Time (ms) |
| 1             | IFNG-BX020     | 125                | PD-1-BX046      | 125                | --              | --                 |
| 2             | --             | --                 | PD-L1-BX067     | 125                | IDO1-BX027      | 125                |
| 3             | --             | --                 | ICOS-BX065      | 125                | PCNA-BX036      | 125                |

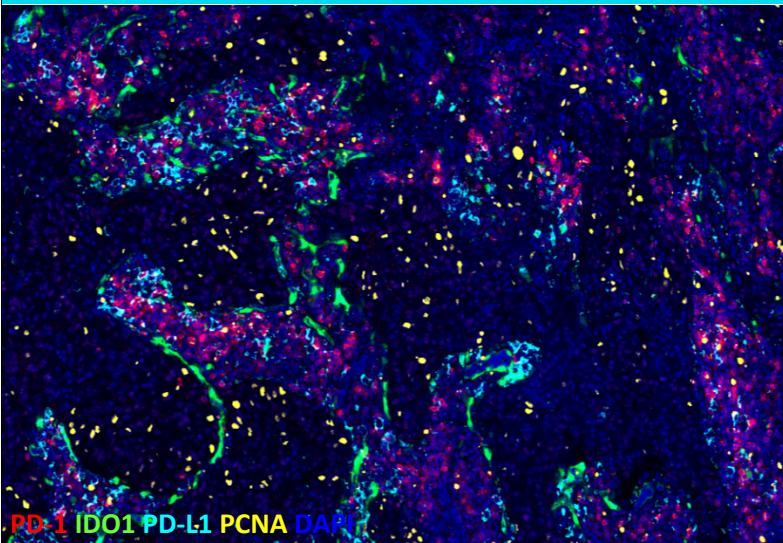
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### HUMAN FFPE TONSIL SECTION



Human FFPE tonsil section was stained with the PhenoCode Discovery Immune Activation and Proliferation Human Protein Module and imaged on the PhenoCycler-Fusion system. Representative imaging regions showing IDO1 (cyan), IFNG (magenta), PCNA (yellow), ICOS (red), PD-1 (orange), PD-L1 (green) and DAPI (blue). Antigen retrieval was performed using AR9 (Akoya, Part# AR900250ML). All antibodies were diluted 1:200.

### HUMAN FFPE LUNG CANCER SECTION



Human FFPE lung cancer section was stained with the PhenoCode Discovery Immune Activation and Proliferation Human Protein Module and imaged on the PhenoCycler-Fusion system. Representative imaging regions showing PD-1 (red), IDO1 (green), PD-L1 (cyan), PCNA (yellow) and DAPI (blue). Antigen retrieval was performed using AR9 (Akoya, Part# AR900250ML). PD-1 and PD-L1 were diluted 1:100, and the rest antibodies were diluted 1:200.