



# A Chromogenic IHC 6-Plex on Tonsil

## DAB, Purple, Red, Yellow, Teal and Silver

Multiplexing enables researchers to gain more information from a single slide.

It also allows investigators do experiments that cannot be done using serial sections, such as co-localization, and determining spatial relationships of various cell types within complex tissues and disease states

This protocol was used to stain tonsil for PD-L1 (DAB), PD-1 (purple), CD68 (red), CD8 (yellow), CD3 (teal) and FOXP3 (silver). These antibodies comprise a standard panel for understanding the immune cell landscape within the tumor microenvironment.

### First Antibody Sequence

- Baking
- Deparaffinization
- Pretreatment
- Cell Conditioning (CC1) 64 min @ 95°C
- DISCOVERY Inhibitor 12 min
- Antibody #1 (PD-L1) 60 min, no heat
- Linking Antibody (anti-Rb HQ) 20 min
- Enzyme conjugate (anti-HQ HRP) 20 min
- DAB

### Second Antibody Sequence

- Antibody denaturation-Extended (CC2) @93°C
- Antibody #2 (PD-1) 32 min @37°C
- Linking Antibody (anti-Ms HQ) 20 min

- Enzyme conjugate (anti-HQ HRP) 20 min
- DISCOVERY Purple H2O2 32 min

### Third Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #3 (CD68) 16min @37°C
- Linking Antibody (anti-Ms NP) 8 min
- Enzyme conjugate (anti-NP AP) 8 min
- DISCOVERY Red-AP 8 min

### Fourth Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #4 (CD8) 16 min @37°C
- Linking Antibody (anti-Rb NP) 12 min
- Enzyme conjugate (anti-NP AP) 12 min
- DISCOVERY Yellow-AP 40 min

### Fifth Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #5 (CD3) 24 min @37°C
- Linking Antibody (anti-Rb HQ) 16 min
- Enzyme conjugate (anti-HQ HRP) 16 min
- DISCOVERY Teal H2O2 16 min
- DISCOVERY Teal Activator

### Sixth Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #6 (FOXP3) 60 min @37°C
- Linking Antibody (anti-Rb HQ) 20 min
- Enzyme conjugate (anti-HQ HRP) 20 min
- Silver 20 min
- Counterstain

# Products Used in This Procedure



Product Name	Roche Number
PD-L1 (sp263)	07494190001
PD-1 (Clone NAT105)	RTU from Cell Marque
CD68 (KP-1)	05278252001
CD8 (sp57)	05937248001
CD3 (sp162)	AbCam
FOXP3 (sp97)	AbCam
DISCOVERY Teal-HRP	08254338001
DISCOVERY Purple-HRP	07053983001
DISCOVERY Yellow-AP	07698445001
DISCOVERY Red-AP	07425333001

There are many ways to approach a 6-plex IHC chromogenic stain, and this is just one example. The DISCOVERY ULTRA is designed to give you the experimental flexibility you need to achieve the best results for your particular tissues and antibodies.

Product Name	Roche Number
DISCOVERY Silver	07053649001
ChromoMap DAB	05266645001
DISCOVERY anti-Rabbit HQ	07017812001
DISCOVERY anti-Mouse HQ	07017782001
DISCOVERY anti-HQ HRP	07017936001
DISCOVERY Anti-Rabbit NP	07425317001
DISCOVERY Anti-Mouse NP	07425309001
DISCOVERY anti-NP AP	07425325001
DISCOVERY CC1	06414575001
DISCOVERY CC2	05424542001

**Visit our website for more information on Ventana Chromogens and how to get started with IHC multiplexing!**

## Chromogen and Detection Reagents

Chemistry	Chromogen	Roche Number
<b>Horseradish Peroxidase</b>	DISCOVERY Red	09576819001
	DISCOVERY Yellow	08502641001
	DISCOVERY Blue	09582690001
	DISCOVERY Purple	07053983001
	DISCOVERY Silver	07053649001
	DISCOVERY Teal	08254338001
	DISCOVERY Green	08478295001
	ChromoMap DAB	05266645001
<b>Alkaline Phosphatase</b>	DISCOVERY Yellow	07698445001
	DISCOVERY Red	07425333001
	ChromoMaP Blue	05266661001
	ChromoMap Red	05266653001

Chromogen	Roche Number
OmniMap Anti-Mouse HRP	05269652001
OmniMap Anti-Rabbit HRP	05269679001
OmniMap Anti-Goat HRP	06607233001
OmniMap Anti-Rat HRP	05891892001
UltraMap Anti-Mouse HRP	05269695001
UltraMap Anti-Rabbit HRP	05269717001
UltraMap Anti-Goat HRP	06607241001
UltraMap Anti-Rat HRP	05891884001

  

Hapten Detection	Roche Number
DISCOVERY Anti-Mouse HQ	07017782001
DISCOVERY Anti-Rabbit HQ	07017812001
DISCOVERY Anti-HQ HRP	07017936001

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For more on multiplexing, visit:



<https://go.roche.com/multiplexing-resources-6-plex>

For more information on Ventana Chromogens for IHC, visit:



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