

# A Chromogenic IHC 4-Plex on Prostate Carcinoma

## DAB, Purple, Yellow and Teal

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 prostate carcinoma for the ERG (endothelial cell marker), Ki67 (cell proliferation marker), p504s (overexpressed in most prostate carcinomas) and VENTANA Basal Cell Cocktail which is an antibody cocktail of anti-p63 (4A4) and anti-keratin (34βE12) mouse monoclonal antibodies.

### First Antibody Sequence

- Baking
- Deparaffinization @69°C
- Pretreatment
- Cell Conditioning (CC1) 64 min @ 95°C
- DISCOVERY Inhibitor 12 min
- Antibody #1 (34BE12+p63) 32 min
- Multimer HRP (OmniMap anti-Ms HRP) 16 min
- DAB

### Second Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #2 (Ki67) 20 min @37°C
- Multimer HRP (OmniMap anti-Rb HRP) 16 min
- DISCOVERY Purple 32 min

### Third Antibody Sequence

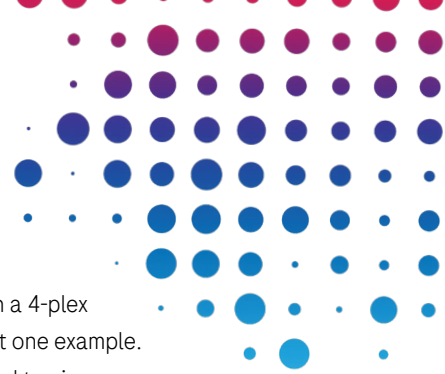
- Antibody denaturation (CC2) @93°C
- Antibody #3 (Erg) 32min @37°C
- Linking Antibody (anti-Rb NP) 12 min
- Enzyme conjugate (anti-NP AP) 12 min
- DISCOVERY Yellow 32 min

### Fourth Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #4 (p504s) 32 min @37°C
- Multimer HRP (OmniMap anti-Rb HRP) 16 min
- DISCOVERY Teal H2O2 16 min
- Teal Activator 16 min
- Counterstain



# Products Used in This Procedure



Product Name	Roche Number
anti-ERG (EPR3864)	06478450001
anti-p504s (SP116)	08035130001
Basal Cell Cocktail	06364497001
anti-Ki-67 (30-9)	05278384001
DISCOVERY Teal-HRP	08254338001
DISCOVERY Purple-HRP	07053983001
DISCOVERY Yellow-AP	07698445001
ChromoMap DAB	05266645001
OmniMap anti-Ms HRP	05269652001
OMNIMAP ANTI-RB HRP	05269679001
DISCOVERY Anti-Rabbit NP	07425317001
DISCOVERY ANTI-NP AP	07425325001
DISCOVERY CC1 (PREDILUTE)	06414575001
DISCOVERY CC2	05279798001

There are many ways to approach a 4-plex 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.

**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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Roche Diagnostics Corporation  
9115 Hague Road  
Indianapolis, Indiana 46256

[diagnostics.roche.com](https://diagnostics.roche.com)

For more on multiplexing, visit:



<https://go.roche.com/multiplexing-resources-4-plex>

For more information on Ventana Chromogens for IHC, visit:



<https://go.roche.com/chromogen-resources-4-plex>

DISCOVERY products are for research use only, not for diagnostic purposes.