



A Chromogenic IHC 3-plex on Breast Carcinoma

Purple, Yellow and Teal

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

It also allows investigators to 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 breast cancer for the biomarkers E-cadherin (E-Cad), Estrogen Receptor (ER) and Progesterone Receptor (PR).

This example is a co-localization experiment. When PR (DISCOVERY HRP-Purple) is detected in the same nucleus with ER (DISCOVERY AP-Yellow), the purple and yellow combine to produce a reddish-brown color - indicating both antigens are found in the same sub-cellular compartment!

There are many ways to approach a 3-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.

First Antibody Sequence

- Deparaffinization
- Cell Conditioning (CC1) 64 min @95°C
- Inhibitor 8 min
- Antibody #1 (PR) 20 min @37°C
- Linking Antibody (anti-Ms HQ) 12 min
- Enzyme conjugate (anti-HQ HRP) 12 min
- DISCOVERY Purple 32 min

Second Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #2 (ER) 16min @37°C
- Linking Antibody (anti-Rb NP) 8 min
- Enzyme conjugate (anti-NP AP) 8 min
- DISCOVERY Yellow-AP 32 min

Third Antibody Sequence

- Antibody denaturation (CC2) @93°C
- Antibody #3 (Ecadherin) 20min @37°C
- Linking Antibody (anti-Ms HQ) 8 min
- Enzyme conjugate (anti-HQ HRP) 8 min
- DISCOVERY Teal H2O2 16 min
- DISCOVERY Teal Activator 16 min
- Counterstain

Products Used in This Procedure



Product Name	Roche Number
E-cadherin (EP700Y)	05973872001
ER (SP1)	05278406001
PR (1E2)	05277990001
DISCOVERY Teal-HRP	08254338001
DISCOVERY Purple-HRP	07053983001
DISCOVERY Yellow-AP	07698445001
DISCOVERY Anti-Rabbit HQ	07017812001
DISCOVERY Anti-Rabbit NP	07425317001
DISCOVERY Anti-HQ HRP	07017936001
DISCOVERY ANTI-NP AP	07425325001
DISCOVERY CC1 (PREDILUTE)	06414575001
DISCOVERY CC2	05279798001

Chromogen and Detection Reagents

Chemistry	Chromogen	Roche Number	Chromogen	Roche Number
Horseradish Peroxidase	DISCOVERY Red	09576819001	OmniMap Anti-Mouse HRP	05269652001
	DISCOVERY Yellow	08502641001	OmniMap Anti-Rabbit HRP	05269679001
	DISCOVERY Blue	09582690001	OmniMap Anti-Goat HRP	06607233001
	DISCOVERY Purple	07053983001	OmniMap Anti-Rat HRP	05891892001
	DISCOVERY Silver	07053649001	UltraMap Anti-Mouse HRP	05269695001
	DISCOVERY Teal	08254338001	UltraMap Anti-Rabbit HRP	05269717001
	DISCOVERY Green	08478295001	UltraMap Anti-Goat HRP	06607241001
	ChromoMap DAB	05266645001	UltraMap Anti-Rat HRP	05891884001
Alkaline Phosphatase	DISCOVERY Yellow	07698445001	Hapten Detection	
	DISCOVERY Red	07425333001	DISCOVERY Anti-Mouse HQ	07017782001
	ChromoMaP Blue	05266661001	DISCOVERY Anti-Rabbit HQ	07017812001
	ChromoMap Red	05266653001	DISCOVERY Anti-HQ HRP	07017936001

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