



A CD3 and CD8 Chromogenic Duplex Protocol

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 with CD3 and CD8 using HQ or NP hapten linker antibodies, anti-hapten enzyme conjugates and DISCOVERY HRP-Purple and AP-Yellow chromogens. This example is a co-localization experiment. CD3 is a T cell marker, and CD8 is a marker of T cell activation. When both antibodies stain the same cell, purple and yellow combine to produce a red color – indicating both antigens are found in the same sub-cellular compartment!

There are many ways to approach a duplex chromogenic stain, and this is just one example. The DISCOVERY ULTRA is designed to be flexible to enable you to achieve the best results for your particular tissues and antibodies.

First Antibody Sequence

- Deparaffinization
- Cell Conditioning (CC1) 64 min @95°C
- Inhibitor 12 min
- Antibody #1 (CD8) 20 min
- Linking Antibody (anti-Rb HQ) 12 min
- Enzyme conjugate (anti-HQ HRP) 12 min
- DISCOVERY Purple 32 min

Second Antibody Sequence

- Antibody denaturation (CC2) @95°C
- Antibody #2 (CD3) 32 min @37°C
- Linking Antibody (anti-Rb NP) 12 min
- Enzyme conjugate (anti-NP AP) 12 min
- DISCOVERY Yellow 32 min
- Counterstain



Products Used in This Procedure



Product Name	Roche Number
DISCOVERY CC1 (PREDILUTE)	06414575001
CD8 (SP57)	Multiple Vendors
DISCOVERY Anti-Rabbit HQ	07017812001
DISCOVERY Anti-HQ HRP	07017936001
DISCOVERY Purple	07053983001
DISCOVERY CC2	05279798001
CD3 (2GV6)	05278422001
DISCOVERY Anti-Rabbit NP	07425317001
DISCOVERY anti-NP AP	07425325001
DISCOVERY AP-Yellow	07698445001

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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