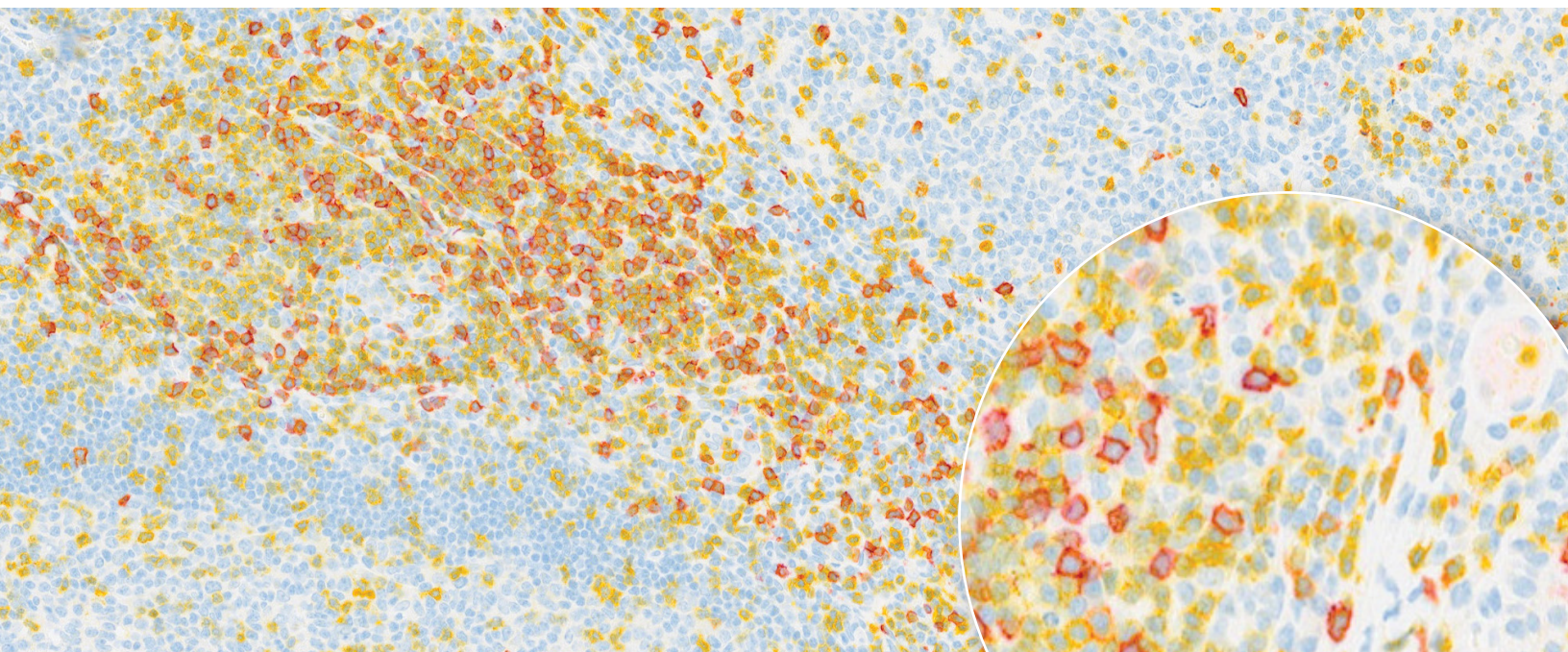




# A CD3 and CD8 Chromogenic Duplex Protocol

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)                | 05937248001  |
| DISCOVERY Anti-Rabbit HQ  | 07017812001  |
| DISCOVERY Anti-HQ HRP     | 07017936001  |
| DISCOVERY Purple          | 07053983001  |
| DISCOVERY CC2             | 05279798001  |
| CD3 (SP162)               | Abcam        |
| 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 |
|-------------------------------|------------------|--------------|--------------------------|--------------|
| <b>Horseradish Peroxidase</b> | 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  |
| <b>Alkaline Phosphatase</b>   | DISCOVERY Yellow | 07698445001  | <b>Hapten Detection</b>  |              |
|                               | 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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