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## Emerald Chromogen for HRP Use in Immunohistochemistry (Organic Resistant Emerald Chromogen for Horseradish Peroxidase Detection)

#Cat: NB-23-00153-1

Size: 18ml for 180 slides

#Cat: NB-23-00153-2

Size: 120ml for 1200 slides

### Intended Use

Emerald Chromogen is provided in ready to use solution. Emerald Chromogen produces an emerald green color precipitate at the site of reaction when it reacts with horseradish peroxidase. Emerald chromogen is resistant to organic solvent which allows the user to dehydrate their slides in graded alcohols and xylene. However it is water soluble so we recommend you limiting the wash steps after emerald staining.

### Kit Components

This product is supplied as ready to use product. Slide tests number based on using 100µL per tissue section.

Emerald Chromogen (RTU)                      18ml (180 slides) / 120ml (1200 slides)

### Recommended Protocol

**We strongly recommend that you counter-stain slides before adding emerald chromogen especially for weak signal to reduce signal loss in wash steps since emerald chromogen is water soluble.**

1. To counter stain before Emerald Chromogen. We recommend the following protocol
  - a) **Counter stain with Hemotoxylin 10 - 30 seconds.**
  - b) **Wash in 3 changes of dH2O 1 minute each.**
  - c) **Blue with PBS or Tris pH7.6 +/- 0.2 for 30 seconds.**
  - d) **Wash in 3 changes of dH2O 1 minute each go to step 2.**

**Warning!** Do not increase incubation times for any steps listed above for counter staining protocol as this will increase the chance of reducing the HRP enzyme activity.

2. Add one or two drops of Emerald chromogen to completely cover tissue section and incubate for 5 minutes. Color development may be monitored under microscope.
3. After proper color development, wash with distilled water 10 seconds for 2-3 times.
4. **Skip this step if you counterstained before Emerald Chromogen!** To counter stain after the application of Emerald Chromogen step follow protocol below.

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- a) **Counterstain with Hemotoxylin 30 seconds.** (We find filtering Hemotoxylin just prior to counter staining with 3MM paper removes precipitant which allows for shorter wash times after counter stain.)
  - b) **Rinse with distill water for 30 seconds 3 times to clear slides.**
  - c) **Blue with PBS or Tris pH 7.6 +/- 0.2 for 30 seconds.**
  - d) **Wash in 3 changes of dH2O 30 to 60 seconds each then go directly to the dehydration steps in #5.** (Note: We recommend 30 seconds for wash since Emerald Chromogen is soluble in water)
5. **Emerald Chromogen is insoluble in organic solvent;** however the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.
- a) 1x 80% Ethanol 20 seconds
  - b) 1x 95% Ethanol 20 seconds
  - c) 3x 100% Ethanol 20 seconds each
  - d) 1x 100% Xylene 20 seconds
  - e) Add 1drop of xylene based mountant and coverslip

**Note:**

If there is green background on the slide, then concentration of primary antibody is too high.

**Storage**

Store at 2-8°C. Do not freeze.

For reference only

For Research Use Only. Not for Diagnostic or Therapeutic Use.