

# Permanent Red Kit for Immunohistochemistry

(Organic Resistant Permanent Red Kit (Improved Formula) for AP Detection)

#Cat: NB-23-00154-1 Size: 120ml for 1200 slides #Cat: NB-23-00154-2 Size: 18ml for 180 slides

#### Intended Use

Permanent Red Kit is provided in three components:

1) Permanent Red Substrate (RTU), 2) Permanent Red Activator (5x), 3) Permanent Red Chromogen (100x) with easy preparation. Permanent Red Kit produces a fresh red color precipitate at the site of reaction when it reacts with alkaline phosphatase. Permanent Red is resistant to organic solvent so it allows the user to dehydrate their slides in graded alcohols and xylene for permanent mount slides.

# **Kit Components**

Component No.	Content	NB-23-00154-2 (180 slides)	<b>NB-23-00154-1</b> (1200 slides)
Reagent 1	Permanent Red Substrate (RTU)	18mL	120mL
Reagent 2	Permanent Red Activator (5x)	3.6mL	12mLx2
Reagent 3	Permanent Red Chromogen (100x)	180µL	1.2mL

#### **Recommended Protocol**

Slides must be washed by distilled water before applying Permanent Red if use PBS as the wash buffer. No need for additional wash if use TBS as the wash buffer.

Prepare ready-to-use permanent red working solution:
Add 200ul of Reagent 2 (Activator) into 1ml of Reagent 1 (Substrate buffer) and mix well. Add 10ul of Reagent 3 into the mixture and mix well. Store at 4°C. Use the mixture within one hour.

[Note: For fewer slides, add  $100\mu$ L of Reagent 2 (Activator) into  $500\mu$ L of Reagent 1 (Substrate buffer) and mix well. Add  $5\mu$ L of Reagent 3 (Chromogen) into the mixture and mix well.]

- 2. Completely cover the tissue section with the mixture and incubate for 5-10 minutes.
- 3. Color development may be monitored under microscope.
- 4. After proper color development, wash with distill water 3 times for 1 minutes each.
- 5. Proceed to counterstaining. Rinse with distilled water to clear slides, then go through dehydration steps in #6.



6. Permanent Red is insoluble in organic solvent; however the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.

**Note:** Please wipe off extra water and air dry slides before dehydration and clear.

- 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 1 drop of xylene based mountant (Cat. No. NeoBio Mount Perm, catalog # NB-23-00156) and coverslip. Press to push the air bubble out.

**CAUTION: DO NOT** dehydrate in xylene longer than 20 seconds! It will erase Permanent Red stain!

7. Permanent Red can be mounted with NeoBio Mount Universal (Aqueous), catalog # NB-23-00157-1 / -2 after counterstain without coverslip. NeoBio Mount Universal will form a crystal clear hard film on the slide after it is dried. Leave slides overnight to be dried completely at room temperature.

## Precaution

Please wear gloves and take other necessary precautions.

### **Storage**

Store at 2-8°C. Do not freeze.

### **Related Products**

Product	Catalog No.	Size
DAB+ Kit (2-component)	NB-23-00148-1 / -2	12ml +240ml / 100ml +2L
DAB Kit (20x Concentrate)	NB-23-00141-1 / -2	100ml / 12ml
AEC kit (20x concentrate)	NB-23-00140	12ml
Fast Red Kit	NB-23-00142	60ml
BCIP/NBT Kit (RTU)	NB-23-00144-1 / -2	100ml / 18ml
NeoBio Mount AQ	NB-23-00155-3	18ml
NeoBio Mount Perm	NB-23-00156	18ml
NeoBio Mount Universal (Aqueous)	NB-23-00157-1 / -2	100ml / 18ml
NeoBio Mount ISH	NB-23-00161-1	100ml
NeoBio Mount Fluo	NB-23-00158-1 / -2	100ml / 18ml
NeoBio Mount Fluo with DAPI	NB-23-00159-1 / -2	100ml / 18ml
NeoBio Mount Fluo with PI	NB-23-00160-1 / -2	100ml / 18ml

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