GBI-Permanent Red Kit

(Organic Resistant GBI-Permanent Red Kit for Alkaline Phosphatase Detection)

Storage: 2-8°C

Catalog No: C13-18 18mL ☐
C13-120 120mL ☐

Intended Use:

GBI-Permanent Red Kit is provided in three components: 1) GBI-Permanent Red Substrate (RTU), 2) GBI-Permanent Red Activator (5x), 3) GBI-Permanent Red Chromogen (100x) with easy preparation. GBI-Permanent Red Kit produces a fresh red color precipitate at the site of reaction when it reacts with alkaline phosphatase. GBI-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:

GBI-Permanent Red Kit is supplied in concentrated form (100x) and the substrate is in ready to use form. Slide tests number based on using 100µL per tissue section.

Component No.	Content	C13-18 (180 slides)	C13-120 (1200 slides)
Reagent 1	GBI-Permanent Red Substrate (RTU)	18mL	120mL
Reagent 2	GBI-Permanent Red Activator (5x)*	3.6mL	24mL
Reagent 3	GBI-Permanent Red Chromogen (100x)	180µL	1.2mL

*Reagent 2 (Activator) will precipitate overtime. This is expected and **will not** affect the quality of the staining.

Recommended Protocol:

If PBS was used as the wash buffer, slides must be washed by distilled water before applying Permanent Red. No additional washes needed if TBS was used as the wash buffer. For best results, first bring **Reagent 1** (substrate) and **Reagent 2** (activator) to room temperature.

1. Shake **Reagent 2** vigorously before adding into **Reagent 1**. ***Reagent 2 will precipitate overtime, but this will not affect its function.*** Using a large pipette (P1000 or larger), add 200ul of **Reagent 2** (Activator) into 1ml of **Reagent 1** (Substrate). ***Any residual precipitate on the pipette tip can be mixed in the Substrate or be discarded.*** Mix until completely clear.
2. Add 12ul of **Reagent 3** into the mixture and mix well. Only use freshly made mixture.
   [Note: For fewer slides, add 100µL of **Reagent 2** (Activator) into 500µL of **Reagent 1** (Substrate buffer) and mix well. Add 6µL of **Reagent 3** (Chromogen) into the mixture and mix well]
3. Completely cover the tissue section with the mixture and incubate for 5-10 minutes.
4. Color development may be monitored under microscope.
5. After proper color development, wash with distill water 3 times for 1 minutes each.
6. Proceed to counterstaining. Rinse with distilled water to clear slides, then go through dehydration steps in #6.
7. **GBI-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. O-Mount, E02-18) and coverslip. Press to push the air bubble out.

   CAUTION: DO NOT dehydrate in xylene longer than 20 seconds! It will erase GBI-Permanent Red stain!
8. Permanent Red can be mounted with Simpo-Mount (Cat. No. E03) after counterstain without coverslip. Simpo-Mount will form a crystal-clear hard film on the slide after it is dried. Leave slides overnight to be dried completely at room temperature.

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Troubleshooting:

Problem	Tips
Reagent 2 (GBI-Permanent Red Activator) has precipitates in the bottle	1. Shake activator bottle until homogenous. 2. Use a large pipette (P1000 or larger) to take 200uL of the activator into 1mL of the substrate. Any residual precipitate on the pipette tip can be mixed in the Substrate or be discarded. The precipitate does not affect the quality of the staining. Mix until completely clear.

Related Products

Product	Catalog No.	Size
DAB+ Kit (2-component DAB)	C09-12 / C09-100	12mL +240mL / 100mL + 2 L
DAB Kit (20x Concentrate)	C02-100 / C02-12	100mL / 12mL
Fast Red Kit (tablets and ready-to-use substrate)	C03-60	12 Tab + 60mL
AEC kit (20x concentrate)	C01-12	12mL
BCIP/NBT Kit (RTU)	C05-100 / C05-18	100mL / 18mL
GB-Mount (Aqueous)	E01-18	18mL
O-Mount (Organic)	E02-18	18mL
Simpo-Mount (Aqueous)	E03-100 / E03-18	100mL / 18mL
ISH Mount (Aqueous) for in situ hybridization	E21-100	100mL
Fluorescent Mounting Medium	E18-100 / E18-18	100mL / 18mL
Fluorescent Mounting Medium with DAPI	E19-100 / E19-18	100mL / 18mL
Fluorescent Mounting Medium with PI	E20-100 / E20-18	100mL / 18mL

Precautions:
Please wear gloves and take other necessary precautions.

Remarks:
For research purposes only.

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