PermaGreen/AP

(Alcohol & Xylene-substitute Compatible)

Catalog No.: K 052-110

Intended Use: As a substrate/chromogen in conjunction with alkaline phosphatase based

immunostaining or in situ hybridization systems.

Introduction: PermaGreen/AP is a substrate-chromogen system designed to be used for either

> IHC or ISH when utilizing alkaline phosphatase. PermaGreen/AP produces a green color that is insoluble in alcohol and xylene substitutes (both aliphatic hydrocarbon and citrus based); therefore sections can be dehydrated in alcohol,

cleared in a xylene substitute, and permanently mounted.

Components: (i) 110mL of buffered solution containing substrate.

(ii) 3mL of concentrated chromogen solution.

(iii) One empty mixing dropping bottle.

Storage: Store at 2-8°C. Do not use beyond expiration date stated on the label.

Aliquot 3mL of PermaGreen/AP Substrate Buffer in a mixing bottle. Add one **Working Solution:**

drop (~20 µl) of PermaGreen/AP Chromogen. Replace tip, mix and allow

solution to reach room temperature before using.

Note: The working chromogen-substrate solution should be used within 1-day of

preparation. Any solution not used during this period should be discarded.

i) After SA-alkaline phosphatase incubation, wash tissue sections with buffer.

ii) Wipe glass, removing excess buffer. Add enough drops of working PermaGreen/AP solution to cover sections.

iii) Incubate for 5-30 minutes at room temperature. For optimal results, observe reaction under microscope for signal development. Once desired signal to noise ratio is achieved, stop reaction by rinsing slides with DI H₂O. NOTE: Increasing incubation temperature to 37°C for 15-20 minutes will increase sensitivity and decrease needed incubation time.

- iv) Counter stain. Hematoxylin provides good contrast. Wash with DI H₂O.
- v) Dehydrate sections in alcohol, clear in a xylene-substitute*, and mount in permanent mounting medium.

*Notes: Use increasing concentrations of Ethanol up to 100% to dehydrate Use xylene-substitute instead of xylene

Alternatively, slides can be air dried (instead of dehydrated/cleared in alcohol and xylene-substitute). After rinsing off counter stain in DI H₂O, leave slides on benchtop for at least 20 minutes to air dry. Then permanently mount.

IVD: For In Vitro Diagnostic Use

DBS will not be held responsible for patent infringement or other violation that may occur with the use of our product



Procedure: