PermaGreen/AP

(Alcohol & Xylene-substitute Compatible)

Catalog No.: K 052

Intended Use: As substrate/chromogen in conjunction with alkaline phosphatase (AP)-based immunostaining or *in situ* hybridization systems.

Introduction: PermaGreen/AP is a substrate-chromogen system designed for either IHC or ISH when using alkaline phosphatase detection. 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) 30mL PermaGreen/AP Substrate Buffer.
(ii) 1mL concentrated PermaGreen/AP Chromogen.
(iii) One empty mixing dropping bottle.

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

Working Solution: Aliquot 3mL of PermaGreen/AP Substrate Buffer in a mixing bottle. Add one drop (~20uL) of PermaGreen/AP Chromogen. Replace tip, mix, and allow solution to reach room temperature before using.
Note: The chromogen-substrate working solution should be used within 1-day of preparation. Any solution not used during this period should be discarded.

Procedure:
i) After SA-AP or AP-polymer incubation, wash tissue sections with buffer.
ii) Wipe glass, removing excess buffer. Add enough drops of PermaGreen/AP working solution to cover sections.
iii) Incubate for 30 minutes at room temperature. For optimal results, observe reaction under microscope for signal development. Once the desired signal to noise ratio is achieved, stop the reaction by rinsing slides with DI H_2O.
NOTE: Increasing incubation temperature to 37˚C for 15-20 minutes will increase sensitivity and decrease needed incubation time.
iv) Counterstain. Hematoxylin or Nuclear Fast Red provides good contrast. Wash with DI H_2O.
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 counterstain in DI H_2O, leave slides on benchtop for at least 20 minutes to air dry, then permanently mount.

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