PermaBlue/AP

(Alcohol & Xylene-substitute Compatible)

Catalog No.: K 051-110

Intended Use: Substrate/chromogen in conjunction with alkaline phosphatase based immunostaining or *in situ* hybridization systems.

Introduction: PermaBlue/AP is a substrate-chromogen system designed to be used for either IHC or ISH when utilizing alkaline phosphatase. PermaBlue/AP produces a vibrant blue color. It is insoluble in alcohol and xylene substitutes (both aliphatic hydrocarbon and citrus based); therefore sections can be dehydrated in alcohol, cleared in xylene substitute*, and permanently mounted.

Components:
(i) 110mL clear PermaBlue/AP Substrate Buffer.
(ii) 3mL concentrated PermaBlue/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 PermaBlue/AP Substrate Buffer in a mixing bottle. Add one drop (~20µl) of concentrated PermaBlue/AP Chromogen solution. Replace tip, mix, and allow solution to reach room temperature before using.
Note: The working PermaBlue/AP chromogen-substrate solution should be used within 25 minutes of preparation. Any solution not used during this period should be discarded.

Procedure:
i) After SA-alkaline phosphatase incubation, wash tissue sections with wash buffer.
ii) Wipe slides, removing excess buffer. Add enough drops of working PermaBlue/AP solution to cover tissue sections.
iii) Incubate for 5-25 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 H_2O.
NOTE: Increasing incubation temperature to 37˚C will increase sensitivity and decrease needed incubation time.
iv) Counter stain. 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 counter stain in DI H_2O, leave slides on benchtop for at least 20 minutes to air dry. Then permanently mount.

IVD: For In Vitro Diagnostic Use

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