



HIGHDEF[®] red IHC chromogen (AP, plus)

Catalog #: ADI-950-141

For Use on Automated Staining Platforms

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTENDED USE

HIGHDEF[®] red IHC chromogen (AP, plus) is a substrate/chromogen system for use with alkaline phosphatase (AP) detection in immunohistochemistry (IHC) or *in situ* hybridization (ISH) run on automated staining instruments as well as manual use.

DESCRIPTION

Traditional formulations for red alkaline phosphatase chromogens are unstable upon mixing. This can be problematic for use in automated slide strainers. HIGHDEF[®] red IHC chromogen (AP, plus) has been formulated for on-slide and on-board mixing for any autostainer. Both components are stable and can be loaded at the beginning of the staining run, for walk away convenience.

HIGHDEF[®] red IHC chromogen (AP, plus) produces a red color, ranging from pink to brilliant dark red. It is insoluble in organic solvents; therefore sections can be dehydrated in alcohol, cleared in xylene (or a xylene-substitute), and permanently mounted. This chromogen substrate system may be used for both automation and manual use.

KIT CONTENTS

Description	30 mL
HIGHDEF [®] red IHC chromogen (AP, plus) Substrate Buffer	30 mL
HIGHDEF [®] red IHC chromogen (AP, plus) Chromogen	30 mL

STORAGE

Store components at 2°-8°C. Chromogen is light sensitive, store away from light. Do not use beyond expiration date stated on the label.

PRECAUTIONS

Wear appropriate personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required. Interpretation of the results is the sole responsibility of the user.

REAGENT PREPARATION FOR MANUAL USE

HIGHDEF® red IHC chromogen (AP, plus) 1X Working Solution: In a clean dark bottle, mix equal volumes of HIGHDEF® red IHC chromogen (AP, plus) Buffer and HIGHDEF® red IHC chromogen (AP, plus) Chromogen in a 1:1 ratio.

MAKE FRESH: WORKING SOLUTION IS STABLE FOR ONLY 20-30 MINUTES.

REAGENT PREPARATION FOR AUTOMATION USE

There is no preparation required for automation use. Reagents can be loaded directly on the instrument for on-slide mixing or on-board mixing.

Protocol	STAINING PROCEDURE:	INCUBATION TIMES
Following alkaline phosphatase incubation, wash tissue sections with wash buffer, then follow protocol of choice:		
On-Slide Mixing (Automation)	HIGHDEF® red IHC chromogen (AP, plus) has been formulated for direct application of the component solutions onto the slide in a specified order: sequential application of substrate buffer first, followed by chromogen in equal volumes with no washing or reagent “blow off” in between. Reversing the order of reagent application will increase background and yield suboptimal results.	Substrate Buffer: 1-5 min Chromogen: 10-20 min
Batch Mode (Automation)	Using Batch Mode on your instrument, wait for machine to notify you when ready, then mix chromogen and substrate-buffer in a 1:1 ratio and load onto instrument.	Working Solution: 10 - 20 min
On Board Mixing (Automation)	Instruments that have on-board mixing capability can load the chromogen and substrate-buffer components independently. Working solution is made mixing reagents 1:1 in on-board mixing station before application to slide.	Working Solution: 10 - 20 min
Manual Use	Mix chromogen and substrate-buffer in a 1:1 ratio and apply directly to slide. Working solution is stable for only 20-30 minutes and should be applied immediately for best results.	Working Solution: 10 - 20 min

COUNTERSTAIN

Counterstain with Hematoxylin for good contrast. Wash with distilled or de-ionized H₂O followed by immuno wash buffer.

Sections can be dehydrated in alcohol, cleared in xylene or xylene substitute and permanently mounted.

MOUNTING

Alternatively, slides can be air dried (instead of alcohol and xylene). After rinsing off counterstain in distilled or de-ionized H₂O, leave slides on benchtop for at least 20 minutes to air dry, then permanently mount or use aqueous mounting media (HIGHDEF[®] IHC mount, Product No. ADI-950-261).

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