



## **HIGHDEF<sup>®</sup> blue IHC chromogen (AP)**

Catalog #: ADI-950-150

**Alcohol & Xylene-Substitute Compatible**

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

## INTENDED USE

Substrate/chromogen in conjunction with alkaline phosphatase (AP)-based immunostaining or *in situ* hybridization (ISH) systems.

## INTRODUCTION

HIGHDEF<sup>®</sup> blue IHC chromogen (AP) is a substrate-chromogen system designed to be used for either immunohistochemistry (IHC) or ISH when utilizing alkaline phosphatase. HIGHDEF<sup>®</sup> blue IHC chromogen (AP) has been formulated for increased stability of the working solution while producing a distinct bright 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. However, we recommend air drying slides and then permanently mounting. This chromogen substrate system may be used for both automation and manual use.

## KIT CONTENTS

Description	30 mL
HIGHDEF <sup>®</sup> blue IHC chromogen (AP) Substrate Buffer	30 mL
HIGHDEF <sup>®</sup> blue IHC chromogen (AP) Chromogen	1 mL
Empty Mixing Bottle	1

## STORAGE

Store at 2°-8°C away from light. Do not use beyond the expiration date stated on the label.

## WORKING SOLUTION

Aliquot 1 mL of HIGHDEF<sup>®</sup> blue IHC chromogen (AP) Substrate Buffer in a mixing bottle. Add one drop (~20 µL) of concentrated HIGHDEF<sup>®</sup> blue IHC chromogen (AP) chromogen solution. Replace tip, mix, and allow solution to reach room temperature before using.

**Note:** The HIGHDEF<sup>®</sup> blue IHC chromogen (AP) chromogen-substrate working solution is light sensitive and should be kept away from light as much as possible. Working solution is stable for up to 6 hours in the dark; any solution not used during this period should be discarded. For optimal staining, use freshly made solution.

Protocol	STAINING PROCEDURE:	INCUBATION TIMES
Following alkaline phosphatase incubation, wash tissue sections with wash buffer, then follow protocol of choice:		
<b>Pre-Mix Working Solution: (Automation)</b>	HIGHDEF® blue IHC chromogen (AP) working solution has a 6-hour stability and can be loaded directly onto instrument as a single solution. Reduce exposure to light to achieve optimal staining. Working solution is applied directly to slide.	<b>Working Solution:</b>  <b>10 - 20 min</b>
<b>On Board Mixing (Automation)</b>	Instruments that have on-board mixing capability can load the chromogen and substrate-buffer components independently. Working solution is made mixing reagents 1:50 in on-board mixing station before application to slide.	<b>Working Solution:</b>  <b>10 - 20 min</b>
<b>Manual Use</b>	Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide.	<b>Working Solution:</b>  <b>10 - 20 min</b>

## COUNTERSTAIN

Counterstain with Hematoxylin or Nuclear Fast Red for good contrast. Wash with distilled or de-ionized H<sub>2</sub>O followed by immuno wash buffer.

## MOUNTING

We recommend air drying slides (instead of dehydrating or clearing in alcohol and xylene-substitute). After rinsing off counterstain in distilled or de-ionized H<sub>2</sub>O, leave slides on benchtop for at least 20 minutes to air dry, then permanently mount.

Alternatively, you may dehydrate sections in increasing concentrations of ethanol up to 100%, clear in a xylene-substitute\*, and mount with a permanent mounting medium (HIGHDEF® IHC mount, Prod. no. ADI-950-261).

**\*Note:** Use xylene-substitute instead of xylene.



# Product Manual

## USE FOR RESEARCH PURPOSES ONLY

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## TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

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