
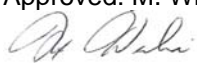
	SOP-BCR-5.15	<b>Immuno-histochemistry Protocol (ALDH Stain)</b>	Author: S. Clouthier, H. Korkaya  Approved: M. Wicha 	Rev: 1.0	Issued: 3/22/10 Revised: 7/17/12
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### 1.0 Purpose

SOP-BCR-5.15 outlines procedures and protocol for ALDH Immunohistochemistry Stain.

### 2.0 Scope

BCR-SOP-5.15 covers all personnel, resources and equipment in the experimental BCR lab.

### 3.0 Materials & Locations

Description	Storage Location
Cycling Water Bath	Located to the right of entrance to 026-328S on the bench near the microwave.
Invitrogen Histostain Plus Kit	Fridge #1 (026-328S-A)(invitrogen cat# 85-8943)
Hematoxylin	
Glycerol	Located in chemical cabinet in 026-328S
1X PBS	Located on bench top near large sink in 026-328S
EtOH, Xylene, H2O	All located in chemical fume hood in 026-328S
Citrate Buffer	Located in 4°C fridge #1 (026-328S-A) (back stock in cold room) Invitrogen cat# 00-5001

### 4.0 Procedure and applicable engineering controls

#### 4.1 Before beginning the staining process:

- take out the citrate buffer from 4°C to allow contents to reach room temperature
- Turn on the cycling water bath and heat to 98°C (this takes about 30 mins)

#### 4.2 Wash slides in fresh xylene solution under chemical hood with three subsequent incubations as follows in order to deparaffine the slides cut from paraffin-embedded tissue sections:

- a: 1<sup>st</sup> wash – 15 minutes
- b: 2<sup>nd</sup> wash – 10 minutes
- c: 3<sup>rd</sup> wash – 10 minutes

#### 4.3 Wash slides in 100% EtOH under the chemical hood

- a: 1<sup>st</sup> wash – 5 minutes
- b: 2<sup>nd</sup> wash – 5 minutes

#### 4.4 Wash slides in running water from the clear/frosted faucet in the sink

- a: 1<sup>st</sup> wash – 5 minutes

- To allow proper washing, let the water gently run and overflow the slide holder for the 5 minute wash. To make sure that the sections are not disturbed by the water, keep the slides on one side of the holder and let the water run in on the other.


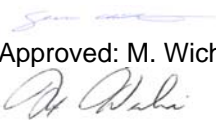
#### 4.5 Incubate slides in citrate buffer for 40 minutes in the 98°C water bath.

- To prevent leaking, tape the top of the slide holder on for the time it will be in the hot water bath. Do not leave the slides in the water bath if the top of the water bath does not close entirely. This will lead to condensation building in the digital panel and malfunctioning of the machine.
- Also utilize the heat gloves to prevent burns, and the small round weights to prevent the cell holder from tipping.

#### 4.6 Remove slide holder from heat bath and set on bench with the top off to allow proper cooling.

- a: >20 minutes recommended to allow proper cooling

#### 4.7 Wash slides in water, exactly as stated in step 4.4 (1 wash – 5 minutes)

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- 4.8** Wipe the slides carefully with a paper towel DO NOT touch tissue sections and transfer them tissue side up on to Styrofoam slide tray.
- 4.9** Cover slides with methanol/3% $H_2O_2$  and incubate for 10 minutes
- 4.10** Wash slides in PBS three times  
 a: 1<sup>st</sup> wash – 2 minutes  
 b: 2<sup>nd</sup> wash – 2 minutes  
 c: 3<sup>rd</sup> wash – 2 minutes
- 4.11** Cover slides with blocking solution (included in kit) for 10 minutes. This solution is found in refrigerator #1 (026-328S-A) in the ALDH staining kit box. It is blue in color. Place a cover slip on each slide during the incubation period. Make sure there are no bubbles that will keep the solution from touching the tissue during this period. Continue to use cover slips for each incubation until step 20.
- 4.12** Drain and wipe the slides with paper towel as in step 4.8
- 4.13** Cover slides with Primary Antibody and incubate for 90 minutes. Antibody dilution is at 1:40 in PBS.
- 4.14** Rinse in PBS two times:  
 a: 1<sup>st</sup> wash – 10 minutes  
 b: 2<sup>nd</sup> wash- 10 minutes
- 4.15** Cover slides with Biotinylated Secondary Antibody (yellow color) from Invitrogen Histostain Plus Kit and incubate for 10 minutes.
- 4.16** Rinse with PBS two times:  
 a: 1<sup>st</sup> wash – 10 minutes  
 b: 2<sup>nd</sup> wash- 10 minutes
- 4.17** Cover with enzyme conjugate (red color) from the same kit and incubate for 10 minutes.
- 4.18** Rinse with PBS three times:  
 a: 1<sup>st</sup> wash – 2 minutes  
 b: 2<sup>nd</sup> wash-- 2 minutes  
 c: 3<sup>rd</sup> wash – 2 minutes
- 4.19** Cover slides with AEC (Invitrogen cat# 00-1111) and incubate for up to 5 minutes if pink color develops earlier.
- 4.20** Rinse well with water
- 4.21** Counter stain the slides with Hematoxylin for 1 minute.
- 4.22** Rinse slides in water
- 4.23** Wash in PBS until sections appear blue (approximately 1 minute)
- 4.24** Rinse slides in water
- 4.25** Add 1 drop of glycerol per slide and mount each section with a cover slip  
 a: Use a 1000uL pipette and cut the end of the pipette tip off  
 b: If bubbles are present, gently press the cover slip onto the glass slide so that bubbles will leave the area. Do not smudge the tissue section while doing this (using the back eraser on a pencil to roll out the air bubbles from under the glass is a good method of doing this).
- 4.26** Place completed slides into a slide booklet and store at room temperature.

**5.0 Applicable References (inc. MSDS and waste disposal where applicable)**

- Please refer to BCR-SOP sections 5.0 for more staining protocols
- Please refer to Invitrogen.com website for more kit specific information.

**6.0 Change Description**

Revision	Date	Description of Change
1.0	7/17/12	Updated room locations