University of Michigan Comprehensive Cancer Center	SOP-BCR-5.4	**Staining for ALDH1 mouse primary antibody**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 07/06/09 Revised: 7/17/12

1.0 Purpose

The purpose of SOP 5.4 is to describe the procedure of staining for paraffin sections ALDH1 mouse primary antibody.

2.0 Scope

SOP 5.4 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	Histostain-plus Kit	Cat# 85-8943 staining kit	Refrigerator #1 (026-328S-A)
2.0	1X PBS	Phosphate Buffered Saline	Cold Storage (026-380C)

4.0 Procedure

4.1 Deparaffine the slides cut from paraffin-embedded Tissue sections.
- 3x xylenes for 15 minutes each (or ON in xylene 1 and 5 minutes each in xylene 2 and 3)
- 2x 100% EtOH for 5 minutes each
- Wash in ddiH_2O for ~5 minutes with running water

4.2 Antigen unmasking (Retrieve): Soak the slides in ready-to-use Citrate Buffer (pH 6.0) for 40 minutes at 98˚C waterbath and let it cool down for about 10 minutes (room temperature) Wash in running distilled H2O for ~15 minutes.

4.3 Submerge slides in PEROXIDASE QUENCHING SOLUTION (cold 3% hydrogen peroxide in methanol) for 10 minutes.

4.4 Wash in 1XPBS for 15 minutes with several changes of PBS.

Use the **Histostain-plus kit (HRP, Broad spectrum CAT# 85-8943)** for ALDH1 Staining:

4.5 Incubate the slides in Serum blocking solution **(Reagent A)** to completely cover tissue Incubate for 10 minutes.

4.6 Blot excess serum from sections.

4.7 Incubate sections in ALDH1 primary antibody diluted in PBS (1:100 dilution) at Room Temperature for 1 hour.

4.8 Wash in fresh 1XPBS for ~15 minutes with several changes of 1XPBS

4.9 Apply enough BIOTINYLATED SECOND ANTIBODY **(Reagent B)** to completely cover the specimen. Incubate for 10 minutes.

4.10 Wash in 1XPBS for 15 minutes with several changes of PBS

4.11 Apply enough ENZYME CONJUGATE **(Reagent C)** to completely cover the cells. Incubate for 10 minutes.

4.12 Wash in 1X PBS for 15 minutes with several changes of PBS.

4.13 Add cold (2-8˚C) AEC Single Solution **(Reagent D)** to slides. Incubate slides at room temperature for 15 minutes.

4.14 Rinse well with distilled water.

4.15 HEMATOXYLIN COUNTERSTAIN (optional):

4.15.1 Immerse slides in a bath of hematoxylin. Length of incubation depends on the strength of hematoxylin used (2 minute). Rinse gently in a distilled water bath for ~15minutes.

4.16 Check the color under the microscope.

4.17 MOUNTING: specimens may be mounted and coverslipped with an aqueous-based mounting medium such as Glycerin (don't dehydrate the slides).

5 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/17/12	CL	Added room locations