University of Michigan Comprehensive Cancer Center	SOP-BCR-5.16	**Immunofluorescence Staining for Paraffin Sections (DAPI, ALDH1, CD24, CD44)**	Author: S. Clouthier Sean McDermott Approved: M. Wicha	Rev: 2	Issued: 6/19/2013 Revised: 11/3/14

1.0 Purpose

The purpose of SOP 5.16 is to provide information on Immunofluorescence staining for paraffin sections (DAPI, ALDH1, CD24, and CD44)

2.0 Scope

SOP 5.16 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Procedure

3.1 Arrange slides in slide holder and take out Citrate Buffer to warm to room temperature (Fridge #1)

3.2 Submerge slides in Xylene for 15 minutes, 3 times. (Move from 1 to 2 to 3).

3.3 Move to 100% EtOH for five minutes, 2 times. (Move from 1 to 2).

3.4 Move to 90-95% EtOH for five minutes, then onto 80% EtOH for five minutes and finally onto 70% EtOH for five minutes.

3.5 Fill slide holder with dd H_2O and transfer slides from EtOH, and put under bubble free regular running water in the sink >5 minutes.

3.6 Antigen unmasking (retrieve): Microwave the slides in ready to use Citrate Buffer (pH 6.0) for 10 minutes, (power level: P7) with lid loosely covered; fill with more Citrate Buffer and microwave another six minutes. Let cool to room temperature.

3.7 Wash in running dd H_2O for >15 minutes. Transfer in 1xPBS to bench.

3.8 Carefully blot dry around the tissue without touching the mounted tissue spot. Use Dako pen to mark around the tissue section (make sure ink is dry before continuing but DO NOT let the tissue section dry-add a drop of PBS with pipettor to the mounted tissue spot after adding pen)

3.9 Incubate the slides in serum blocking solution (Reagent A) from the Histostain-Plus kit (Invitrogen 85-8943)-cover the tissue mounted spot only with pipettor-for **10 minutes** at room temperature.

3.10 Blot excess serum from the slides.

3.11 Add 1º Antibody

- **CD44** (Mouse) (Thermo Scientific MS-668-R7)-in fridge
- **READY TO USE**
- Incubate **overnight** in cold room in a slide box lined with moist paper towels to keep the inside of the box moist while incubating overnight

3.12 **Next day: TURN OFF LIGHTS**. Proceed with the remaining steps in the **DARK**.

3.13 Wash slides with room temperature 1xPBS, 3 times, five minutes each wash.

3.14 Add 2º Antibody

- **ALEXAFLUOR 488** (Anti-Mouse) (Invitrogen A11001)-in freezer (it can be either goat or rabbit host, the source/host does not matter, just that it is anti whatever was used as primary, so if primary is mouse, then secondary must be anti-mouse)
- **1:200** in PBS
- Incubate for **20 minutes** at room temperature

3.15 Wash slides with room temperature 1xPBS, 3 times, five minutes each wash.

3.16 Incubate the slides in serum blocking solution (Reagent A) from the Histostain-plus Kit (Invitrogen 85-8943) for **30 minutes** at room temperature.
- Blot excess serum from the slides.

3.17 Add next 1º Antibody
- **CD24** (Mouse) (Neomarkers MS-1279-P1)
- **1:50** in PBS
- Incubate for **1 hour** at room temperature.

3.18 Wash slides with room-temperature 1xPBS, 3 times for five minutes each wash.

3.19 Add 2º Antibody
- **ALEXAFLUOR 647** (Anti-Mouse) (Jackson 715-606-150)
- **1:200** in PBS
- Incubate for **20 minutes** at room temperature.

3.20 Wash slides with room temperature 1xPBS, 3 times. If not adding ALDH stain, move onto step 3.26!

3.21 Incubate the slides in serum blocking solution (Reagent A) from the Histostain-plus kit (Invitrogen 85-8943) for **30 minutes** at room temperature.
- Blot excess serum from the slides.

3.22 Add 1º Antibody
- **Anti-ALDH1A1** (Rabbit) (Abcam ab52492)
- **1:50** in PBS
- Incubate **overnight** at room temperature.

3.23 Wash slides with room temperature 1xPBS, 3 times.

3.24 Add 2º Antibody
- **ALEXAFLUOR 546** (Anti-Rabbit) (Invitrogen A11035)
- **1:200** in PBS
- Incubate for **20 minutes** at room temperature.

3.25 Wash slides with room-temperature 1xPBS, 3 times.

3.26 Mounting:
- **Mount slides with Invitrogen ProLong Gold Antifade Reagent including DAPI** (Cat # P-36931)
- Use the minimum amount necessary for mounting → results in better picture quality (about one drop, about 20-30 uL per drop) then carefully add cover slide

3.27 Storage:
- Store slides overnight in the DARK at room temperature

3.28 Pictures:
- Analyze slides and capture photos after overnight storage

3.29 Long-Term Storage:
- Wrap slides in foil and store at 4º

4.0 Applicable References

4.1 SOP 4.28 Preparation of Citrate Buffer for IHC

5.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/25/14	TL	Stain with CD44 first b/c shows better when incubates overnight
2.0	11/3/14	TL	Added applicable reference