

	SOP-BCR-5.8	<b>Staining Procedure for Paraffin sections (Factor VIII rabbit primary antibody (NeoMarkers)) 8-30-07</b>	Author: S. Clouthier  Approved: M. Wicha 	Rev: 1.0	Issued: 09/24/98 Revised: 07/17/12
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### 1.0 Purpose

The purpose of SOP 5.8 is to provide instructions on how to stain for paraffin sections using Factor VIII rabbit primary antibody.

### 2.0 Scope

SOP 5.8 is intended to cover all resources, personnel and equipment in the BCR laboratory.

### 3.0 Materials

No.	Name	Description	Storage Location
1.0	Citrate Buffer	For immunohistochemical staining	Fridge #1/Cold Storage (026-328S-A/026-380C)
2.0	Peroxidase Quenching Solution	Staining Agent	Freezer #2 (026-328S-A)
3.0	Serum blocking Solution	Reagent A in AEC kit	Fridge #1 (026-328S-A)
4.0	Biotinylated second antibody	Reagent B in kit	Fridge #1 (026-328S-A)
5.0	Enzyme Conjugate	Reagent C in kit	Fridge #1 (026-328S-A)
6.0	AEC Single Solution	Reagent D in kit	Fridge #1 (026-328S-A)

### 4.0 Procedure

4.1 Deparaffine the slides cut from paraffin-embedded Tissue sections.

- 3x xylenes for 10 minutes each.
- 2x 100% EtOH for 5 minutes each.
- Wash in dH<sub>2</sub>O for 5 minutes on a rocker plate.
- When conducting Step 1, please set the water bath fo 98° C

4.2 Antigen unmasking (Retrieve): Soak the slides in heated (98° C water bath) ready-to-use Citrate Buffer (pH 6.0) for 40 minutes. Take the slides out together with the buffer from the water bath and let it cool down to room temperature (takes more than 20 minutes). Wash in dH<sub>2</sub>O for 5 minutes.

4.3 Submerge slides in Peroxidase Quenching Solution (cold 3% hydrogen peroxide in methanol) for 10 minutes.




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

4.5 Start to use the Histostain®-Plus Kit (AEC kit from Zymed Lab, Cat. 85-9943)

4.6 Add enough Serum blocking solution (Reagent A) to completely cover tissue. Incubate for 10 minutes then drain or blot off solution. **DO NOT RINSE.**

#### 4.7 PRIMARY ANTIBODY

- Tap off excess Serum blocking solution and wipe slides as before. Apply enough optimally diluted primary antibody (1:100 dilution of Factor VIII primary antibody in 1x PBS) to cover cells. Incubate 1 hour at room temperature. (ALDH1 – 1:50 dilution)
- Wash in fresh 1x PBS for > 15 minutes with several changes of 1x PBS.

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- 4.8 Apply enough BIOTINYLATED SECOND ANTIBODY (Reagent B) to completely cover the cells. Incubate for 10 minutes.
- 4.9 Wash in 1x PBS for 15 minutes with several changes of PBS.
- 4.10 DO NOT EQUILIBRATE AEC *Single Solution* TO ROOM TEMPERATURE BEFORE USE! Add cold (2-8°C) AEC *Single Solution* (Reagent D) to slides. Immediately return AEC *Single Solution* back to 2-8°C storage. Incubate slides at room temperature for 10-15 minutes (Need to observe under microscope).
- 4.11 Rinse well with distilled water.
- 4.12 HEMATOXYLIN COUNTERSTAIN
- 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 > 15 minutes.
  - Check the color under microscope.
- 4.13 MOUNTING
- Specimens may be mounted and cover slipped with an aqueous-based mounting medium such as Glycerin (don't dehydrate the slides)
  - Can store the slides up to one year.

## 5.0 Applicable References

## 6.0 Change Description

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