University of Michigan Comprehensive Cancer Center	SOP-BCR-5.13	**Staining for Paraffin section (scribble (c-20) sc-11049, santa cruz) w/SuperPicTure polymer kit (for goat primary antibody)**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 09/24/98 Revised: 07/17/12

1.0 Purpose

The purpose of SOP 5.13 is to provide instructions on how to stain for Paraffin sections (scribble (c-20) sc-11049, santa cruz) with SuperPicTure polymer kit for goat primary antibodies.

2.0 Scope

SOP 5.13 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		Fridge #1/cold room (026-328S-A/026-380C)
2.0	Peroxidase quenching solution	Cold 3% hydrogen peroxide in methanol	
3.0	1x PBS	Phosphate buffering saline	Cold Storage (026-380C)
4.0	HRP polymer conjugate	Reagent A in SuperPicTure polymer kit	Fridge #1 (026-328S-A)

4.0 Procedure

4.1 Always shake everything before using!

4.2 Glass dish needs to be moist, wet paper towel

4.3 Deparaffine the slides cut from paraffin-embedded Tissue sections

- 3x xylenes for > 15 minutes each
- 2x 100% EtOH for 5 minutes each
- Wash in d H20 for > 5 minutes in running water.

4.4 Antigen unmasking (Retrieve): Soak the slides in ready to use Citrate Buffer (PH 6.0) and heated at 98° C for 40 minutes and let it cool down for about 10 minutes (room temperature). Wash in dH20 for > 15 minutes.

4.5 Submerge slides in Peroxidase quenching solution for 10 minutes.

4.6 Wash in PBS for 15' with several changes of PBS.

4.7 Apply blocking serum as mentioned. (horse serum 1%)

4.8 Start to use the SuperPicTure polymer kit (Zymed Lab, Cat. 87-9363)

4.9 PRIMARY ANTIBODY: Tap off excess pBS and wipe slides as before. Apply enough optimally diluted primary antibody (**1:100** dilution of **Scribble** primary antibody in 1x PBS) to cover the tissue. Incubate for 1 hour at RT

4.10 Wash in fresh 1x PBS for > 10 minutes with > 2 changes of 1x PBS. For < 4 slides: 1 wash. For > 4 slides do 3 washes.

4.11 Apply enough HRP polymer conjugate (Reagent A) to completely cover the tissue. Incubate for ten minutes. Make sure there are no bubbles! Remove with pipette.

4.12 Wash in fresh 1x PBS for > 10 minutes with >2 changes of 1x PBS.

4.13 Add 1 drop of reagent B1, 2 drops of reagent B2 and 1 drop of reagent B3 to 1 mL distilled water. Mix well and protect from light. Add enough to cover the tissue and incubate slides at room temperature for 5 (7) minutes. (need to observe under microscope).

4.14 Rinse well with distilled water.

4.15 HEMATOXYLIN COUNTERSTAIN (optional): used for staining the nucleus.

- Immerse slides in a bath of hematoxylin. Length of incubation depends on the strength of hematoxylin used (1 minute). Rinse gently in a distilled water bath for > 10 minutes. Check the color under microscope.

4.16 MOUNTING: Specimens may be mounted and cover slipped with an aqueous-based mounting medium such as Glycerin (don't dehydrate the slides).

4.17 Can store the slide up to one year.

5.0 Applicable References

6.0 Change Description

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