University of Michigan Comprehensive Cancer Center	SOP-BCR-5.7	**Staining procedure for Tissue Sections (*Goat* Primary Antibody) by using RTU Vectastain Universal Quick Kit**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 09/24/98 Revised: 7/17/12

1.0 Purpose

The purpose of SOP 5.7 is to provide instructions on how to stain for tissue sections (*Goat* primary antibody) by using RTU Vectastain Universal Quick Kit.

2.0 Scope

SOP 5.7 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	1x PBS	Phosphate buffering saline	Cold Storage (026-380C)
2.0	Peroxidase quenching solution	(3% hydrogen peroxidase in methanol)	
3.0	RTU Biotinylated pan specific universal secondary antibody	Binds to primary antibody	Fridge #1 (026-328S-A)
4.0	Streptavidin/peroxidase complex reagent	Binds to any biotinylated antibody	Fridge #1 (026-328S-A)
5.0	Peroxidase substrate solution	Staining Agent	Freezer #2 (026-328S-A)

4.0 Procedure

4.1 Deparaffine the slides cut from paraffin-embedded Tissue section.
- 3x xylenes for > 15 minutes
- 2x 100% EtOH for 5 minutes each
- Wash in dH_20 for > 5 minutes in running water.

4.2 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 (to room temperature). Wash in running dH_20 for > 15 minutes.

4.3 Submerge slides in peroxidase quenching solution (3% hydrogen peroxide in methanol) for 10 minutes.

4.4 Wash slides in 1x PBS for 5 minutes

4.5 Incubate the slides in blocking serum for 10 minutes.

4.6 Blot excess serum from sections.

4.7 Incubate sections in primary antibody diluted in PBS containing 1.5% blocking serum for 1 hour (For **Scribble: 1:100**)

4.8 Wash slides for 5 minutes in PBS.

4.9 Incubate sections in RTU biotinylated pan specific universal secondary antibody for 10 minutes.

4.10 Wash sections for 5 minutes with PBS.

4.11 Incubate sections in ready-to-use streptavidin/peroxidase complex reagent for 5 minutes.

4.12 Wash sections for 5 minutes with PBS.

4.13 Protect from the light!

4.14 Incubate sections in peroxidase substrate solution (DAB Substrate Kit for peroxidase) until desired stain intensity develops. Immediately before use, prepare the substrate solution as follows.

- To 5.0 mL of distilled water, add 2 drops of Buffer Stock Solution and mix well.
- Add 4 drops of DAB Stock Solution and mix well.
- Add 2 drops of the Hydrogen Peroxide Solution and mix well.
- If a gray-black stain is desired, add 2 drops of the Nickel Solution and mix well.
- Incubate tissue sections with the substrate at room temperature until suitable staining develops. Development times should be determined by the investigator but generally 2-10 minutes provides good staining intensity.

4.15 Wash sections in dH_20 for 5 minutes.

4.16 Counter stain, clear and mount. (Optional).

5.0 Applicable References

6.0 Change Description

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