University of Michigan Comprehensive Cancer Center	SOP-BCR-5.10	**Staining Procedure for Goat Primary Antibody by using Zymed kit (Cat 85-8943) BUT using DAB substrate instead of AEC**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 09/24/98 Revised: 7/17/12

1.0 Purpose

The purpose of SOP 5.10 is to provide instructions on the staining procedure for Goat primary antibody using the Zymed kit with a DAB substrate instead of AEC.

2.0 Scope

SOP 5.10 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	Peroxidase Quenching Solution	Staining Agent	Cold Storage (026-380C)
2.0	Primary Antibody	Scribble	Fridge #1 (026-328S-A)
3.0	Secondary Antibody Polyclonal Rabbit Anti-goat Immunoglobulins/ Biotinylated	Binds to Primary Antibody	Fridge #1 (026-328S-A)
4.0	Enzyme Conjugate	Reagent C from kit	Fridge #1 (026-328S-A)
5.0	1x PBS	Phosphate Buffering 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
- 2x 100% EtOH for 5 minutes each
- Wash in dH_20 for > 15 minutes.

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 (room temperature). Wash in running dH_20 for > 15 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 PRIMARY ANTIBODY: Tap off excess PBS and wipe slides bottom. Apply enough optimally diluted primary antibody (**1:100** dilution of **scribble** primary antibody in 1x PBS) to cover cells. Incubate 1 hour at RT. Wash in fresh 1x PBS for > 15 minutes with several changes of1x PBS.

4.6 Apply enough SECONDARY ANTIBODY Polyclonal Rabbit Anti-goat Immunoglobulins/Biotinylated (DAKO, Cat E0466) (**1:200** dilution in 1x PBS) to completely cover the tissue. Incubate for 25 minutes.

4.7 Wash in 1x PBS for 15 minutes with several changes of PBS.
4.8 Apply enough ENZYME CONJUGATE (Reagent C from the kit) to completely cover the tissue. Incubate for 10 minutes.
4.9 Wash in 1x PBS for 15 minutes with several changes of PBS.
4.10 Prepare DAB substrate and incubate slides with DAB substrate at room temperature for 10 minutes (need to observe under microscope).
4.11 Rinse well with distilled water.
4.12 HEMATOXYLIN COUNTERSTAIN (optional)

- 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 > 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 cells!)
- 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