

	SOP-BCR-5.6	Staining Procedure for Tissue Sections (BRCA1 mouse primary antibody (MS110)) by using fluorescence FITC-conjugated anti-mouse secondary antibody	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 5.6 is to provide instructions on the staining procedures for tissue sections (BRCA1 mouse primary antibody (MS110)) using fluorescence FITC-conjugated anti-mouse secondary antibody.

2.0 Scope

SOP 5.6 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 (026-328S-A)
2.0	1x PBS	Phosphate Buffering Saline	Cold Storage (026-380C)
3.0	Blocking Serum	In Zymed Kit	Fridge #1 (026-328S-A)
4.0	MS110	Primary Antibody	Fridge #1 (026-328S-A)
5.0	FITC-conjugated donkey anti-mouse	Secondary Antibody	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 dH₂O for 5 minutes on a rocker plate.
- When conducting Step 1, please set the water bath to 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 dH₂O for 5 minutes.

4.3 Wash slides in 1x PBS about 3times for 3 minutes each.

4.4 Incubate the slides in blocking serum (from the Zymed Kit, not goat serum) for 10 minutes.

4.5 Blot excess serum from sections.

4.6 Incubate sections in primary antibody **MS110** diluted in PBS (**1:100 dilution**) at 4° C overnight.

4.7 Wash slides in 1x PBS three times for three minutes each.

4.8 Incubate sections in FITC-conjugated donkey anti-mouse secondary antibody from Jackson Laboratory diluted in 1x PBS (**1:100 dilution**) for 20 minutes.

4.9 Wash sections in 1x PBS three times for three minutes each.

4.10 Rinse the slides gently with distilled water from a wash bottle (do not focus flow directly on tissue).

4.11 Mount with the mounting medium with DAPI. Check the color under microscope.

5.0 Applicable References

6.0 Change Description

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