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|  | SOP-BCR-5.3 | Staining on Collagen Plates | Author: S. Clouthier  Approved: M. Wicha  | Rev: 1.0 | Issued: 09/24/98 Revised: 7/17/12 |
|---|-------------|------------------------------------|--|-------------|--------------------------------------|

1.0 Purpose

The purpose of SOP 5.3 is to describe staining procedures for cells on collagen plates.

2.0 Scope




SOP 5.3 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 buffered saline | Cold Storage (026-380S) |
| 2.0 | H ₂ O ₂ | Hydrogen Peroxide | Chemical Cabinet (026-314S) |
| 3.0 | CH ₃ OH | Methanol | Chemical Cabinet (026-314S) |
| 4.0 | Serum Blocking solution | Blocking Solution | |
| 5.0 | Secondary Antibody | Binds to primary antibody | |
| 6.0 | Enzyme Conjugate | Reagent C in AEC kit | |
| 7.0 | AEC single solution | | Door of Fridge #1, brown bottle (026-328S-A) |

4.0 Procedure

- 4.1 Set paper towel down by sink
- 4.2 Dump medium off of collagen plates into sink.
 - Can leave the lids off
- 4.3 Add PBS to each plate then pour it out
- 4.4 Add cold Methanol and incubate back into the freezer,
 - Make sure to keep the lid loose.
 - While waiting, make a one part 3% Hydrogen peroxide to nine parts methanol mixture.
- 4.5 Discard the methanol
- 4.6 Pour Peroxidase mixture into each plate and leave on counter
 - Pour out after 10 minutes
- 4.7 Wash with PBS and leave in the plates for 2 minutes
 - Do this three times total
- 4.8 Add 0.5 mL SERUM BLOCKING SOLUTION (goat serum) and incubate for 10 minutes on counter.
- 4.9 While it is incubating, prepare PRIMARY ANTIBODY. Obtain it from the 1st freezer in the box labeled more ab. Do a 1:20 dilution with PBS.
 - Ex) 150 µL cytokeratin 18 for 3 mL PBS
- 4.10 Once 10 minute incubation of Serum Blocking Solution is over, dump out serum.
- 4.11 DO NOT RINSE, and add 0.5 mL of the PRIMARY ANTIBODY
 - Let sit on the counter for 50 minutes occasionally swirling it.
- 4.12 Once 50 minutes is over, wash with PBS three times and let it sit for two minutes in between as before.
 - But put the plates on the mixer on a really slow speed so they are washed well.

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- 4.13 Add 0.5 mL SECONDARY ANTIBODY (reagent B) to each plate and swirl around. Let incubate on the counter for 20 minutes.
- 4.14 Rinse with PBS for two minutes, three times.
- 4.15 Add 0.5 mL of Enzyme Conjugate to each plate.
 - Incubate for 10 minutes on counter.
- 4.16 Rinse with PBS for two minutes, three times.
- 4.17 **TURN OFF THE LIGHTS BEFORE NEXT STEP**
- 4.18 Add 0.5 mL of AEC single solution.
- 4.19 The colonies should be tinted pinkish red ~ 5 minutes.

5.0 Applicable References

6.0 Change Description

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