

	SOP-BCR-5.1	Staining	Author: S. Clouthier  Approved: M. Wicha 	Rev: 1.0	Issued: 09/24/98 Revised: 7/17/12
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1.0 Purpose

The purpose of SOP 5.1 is to provide instructions for staining

2.0 Scope

SOP 5.1 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	Serum blocking solution	Reagent A	Fridge #1 (026-328S-A)
3.0	Second Antibody	Reagent B	Fridge #1 (026-328S-A)
4.0	CT14		Freezer #1 (026-328S-A)
5.0	Alkaline Phosphate conjugate	Reagent C	Fridge #1 (026-328S-A)
6.0	Chromagen	Stain	

4.0 Procedure

- 4.1 Rinse off the first stain with PBS for two minutes, three times.
- 4.2 Add 0.5 mL of SERUM BLOCKING SOLUTION (blue- reagent A) to each plate and incubate for 10 minutes. The second kit looks just like the first except only 3 bottles inside (A, B and C)
- 4.3 During 10 minute incubation, prepare PRIMARY ANTIBODY
 - 150 µL CT14 (in box in freezer #1 (026-328S-A) labeled more ab) and 3 mL PBS
- 4.4 After 10 minutes, pour out serum but DO NOT RINSE.
- 4.5 Add 0.5 mL of PRIMARY ANTIBODY to each plate.
- 4.6 Incubate on counter for 60 minutes.
- 4.7 After 60 minutes, dump out primary antibody and rinse with PBS for two minutes, three times.
- 4.8 Apply 0.5 mL of SECOND ANTIBODY (Reagent B) to each plate and incubate for 20 minutes on the counter.
- 4.9 After 20 minutes, dump out and rinse 3 times with PBS for 2 minutes.
- 4.10 Add 0.5 mL of ALKALINE PHOSPHATASE CONJUGATE (Reagent C) and incubate for 20 minutes on counter.
- 4.11 During 20 minute incubation, prepare the chromogen.
 - Add 100 µL of stock to 5 mL of solution labeled ©
- 4.12 After 20 minutes, dump out and rinse 3 times with PBS for 2 minutes.
- 4.13 Add 0.5 mL of chromogen mix to each plate and watch the colonies change colors (purple).
Wait <5, takes longer!

5.0 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/17/2012	CL	Added room locations